

ANNALS OF BOTANY

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CONTENTS.

No. CXXXVII, January, 1921.

	PAGE
BEWS, J. W.—Some General Principles of Plant Distribution as illustrated by the South African Flora	1
HODAY, M. G.—Anatomy of the Ovule and Seed in <i>Gnetum Gnetum</i> , with Notes on <i>Gnetum funiculare</i> . With Plate I and five Figures in the Text	37
STOKES, MARIE CARMICHAEL.—The Missing Link in <i>Osmundites</i> . With Plate II and one Figure in the Text	55
TUTTING, E. M.—On the Pollination Mechanism of <i>Incarvillea Delavayi</i> , Franch. With three Figures in the Text	63
TORREY, K. E.—Telephragmoxyton and the Origin of Wood Parenchyma. With Plate III and three Figures in the Text	73
DAVEY, DOROTHY M.—Some Observations on the Life-history of <i>Nectria galligena</i> , Bres. With Plates IV and V	79
GREGORY, F. G.—Studies in the Energy Relations of Plants. I. The Increase in Area of Leaves and Leaf Surface of <i>Cucumis sativus</i> . With fifteen Figures in the Text	93
WORMALD, H.—On the Occurrence in Britain of the Ascigerous Stage of a 'Brown Rot' Fungus. With Plates VI and VII	115

NOTES.

WALSDEN, H.—The Isolation of the Organism causing Crown Gall on <i>Chrysanthemum frutescens</i> in Britain	137
WILLIAMSON, H. S.—A New Method of preparing Sections of Hard Vegetable Structures	159

No. CXXXVIII, April, 1921.

CAMPBELL, DOUGLAS HIGHTON.—The Gametophyte and Embryo of <i>Botrychium obliquum</i> , Muhl. With Plate VIII and eleven Figures in the Text	141
CHACKRUEEN, KATHLEEN B., and HARRISON, J. W. HESLOP.—The Status of the British Rose Forms as determined by their Cytological Behaviour. With Plates IX and X and five Figures in the Text	159
SMITH, T. O., and BUTLER, O.—Relation of Potassium to Growth in Plants	189
JEFFERY, EDWARD CHARLES, and TORREY, RAY ETHAN.—Transitional Herbaceous Dicotyledons. With Plates XI-XIII and five Figures in the Text	227
HOLMES, M. G.—A Contribution to the Study of Water-conductivity in Sycamore Wood. With thirteen Figures in the Text	251
SHERKIZ, WILLIAM.—Observations on some Physical Properties of Protoplasm by Aid of Microdissection. With one Figure in the Text	269

NOTES.

SHOEN, R.—Note on the Presence of a 'Tent-pole' in the Seed of <i>Cephalotaxus pedunculata</i> . With two Figures in the Text	297
WELLSFORD, L. J.—Division of the Nuclei in <i>Synchytrium endobioticum</i> , Perc. With one Figure in the Text	298

No. CXXXIX, July, 1921.

ABER, A. MRS.—The Leaf Structure of the <i>Indaceae</i> , considered in Relation to the Phyllode Theory. With sixty-six Figures in the Text	301
DAVEY, C.—Studies in the Physiology of Parasitism. VI. Infection by <i>Sclerotinia Libertiana</i> . With Plate XIV	337

	PAGE
MOTTIER, DAVID M.—On Certain Plastids, with Special Reference to the Protein Bodies of <i>Zea</i> , <i>Ricinus</i> , and <i>Conopholis</i> . With Plate XV	349
GATES, R. RUGGLES, and REES, E. M.—A Cytological Study of Pollen Development in <i>Lactuca</i> . With Plates XVI-XIX	365
DASTUR, JEHANGIR FARDUNJI.—Cytology of <i>Tilletia Triticici</i> , (Bjerk.) Wint. With Plate XX and nine Figures in the Text	399
CUTTING, E. M.—Observations on Variations in the Flowers of <i>Stachys sylvatica</i> , Linn. With five Figures in the Text	409
BROWNE, ISABEL M. P.—A Fourth Contribution to our Knowledge of the Anatomy of the Cone and Fertile Stem of <i>Equisetum</i> . With Plate XXI and twelve Figures in the Text	427
NOTES.	
CHANDLER, M. E. J.—Note on the Occurrence of <i>Sequoia</i> in the Hendon Beds of Hordwell, Hants	457
HORNE, ARTHUR S.—Phloem Necrosis (Brown Bast Disease) in <i>Hevea brasiliensis</i>	457

No. CXL, October, 1921.

HOLDEN, H. S. and DANIELS, MARGARET E.—Observations on the Anatomy of Teratological Seedlings. IV. Further Studies on the Anatomy of Atypical Seedlings of <i>Impatiens Roylei</i> , Walp. With ninety-seven Figures in the Text	461
WILLIS, J. C.—Endemic Genera of Plants in their Relation to Others. With six Diagrams in the Text	493
GUPPY, H. P.—The Testimony of the Endemic Species of the Canary Islands in Favour of the Age and Area Theory of Dr. Willis	513
TAYLOR, NORMAN.—Endemism in the Bahama Flora. With one Map in the Text	523
BRENCHLEY, WINIFRED E., and JACKSON, VIOLET G.—Root Development in Barley and Wheat under Different Conditions of Growth. With four Figures in the Text	533
WATERHOUSE, W. L.—Studies in the Physiology of Parasitism. VII. Infection of <i>Barberis vulgaris</i> by Sporidia of <i>Puccinia graminis</i> . With nineteen Figures in the Text	557
HATFIELD, E. J.—Anatomy of the Seedling and Young Plant of <i>Macrozamia Fraseri</i> . With Plate XXII and eight Figures in the Text	565
THODAY, D.—On the Behaviour during Drought of Leaves of two Cape Species of <i>Passerina</i> , with some Notes on their Anatomy. With thirteen Figures in the Text	585
WILLIAMS, J. LLOYD.—The Gametophytes and Fertilization in <i>Laminaria</i> and <i>Cordia</i> . Preliminary Account	603
EDWARDS, W. N.—Fossil Coniferous Wood from Kerguelen Island. With Plate XXIII and four Figures in the Text	609

Some General Principles of Plant Distribution as illustrated by the South African Flora.

BY

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INTRODUCTION.

THE geographical distribution of plants, which has been described by Darwin (20), in one of his letters to Sir Joseph Hooker, as 'that grand subject, that almost keystone of the laws of creation', has been studied from many different standpoints, and, with the increasing interest in plant ecology, is now attracting more and more attention. Darwin's own position is summarized in a single sentence in the 'Origin of Species' (16, p. 35c): 'If the difficulties be not insuperable in admitting that in the long course of time all individuals of the same species, belonging to the same genus, have proceeded from one source, then all the grand leading facts of geographical distribution are explicable on the theory of migration, together with subsequent modification and the multiplication of new forms.' The general progress of the study of plant distribution since the publication of the 'Origin of Species' has been dealt with by Thiselton-Dyer (20), from whose paper a few extracts will be taken. 'If Darwin laid the foundations, the present fabric of botanical geography must be credited to Hooker. It was a happy partnership. The far-seeing generalizing power of the one was supplied with data and checked in conclusions by the vast detailed knowledge of the other.' Hooker's views were given in the 'Introductory Essay to the Flora of Tasmania' (27) and in the 'Distribution of Arctic Plants'—publications which were, according to Thiselton-Dyer, 'only less epoch-making than the "Origin" itself'. After Darwin, Wallace carried on the task of investigation, and his views are given in his well-known work 'Island Life' (35) and as late as 1911 in 'The World of Life' (36).

Both Wallace and Darwin objected to invoking geographical change as a solution of every difficulty. They believed in the general stability and permanence of our continental areas, while admitting 'wonderful and repeated changes in detail' (35, p. 101). Their views in this respect received great support as the result of the *Challenger* expedition, which furnished proof that the floor of the ocean basins has no real analogy

among the sedimentary formations which form most of the framework of the land. Hooker, however (27), was much more inclined than either Darwin or Wallace to find a way out of difficulties by supposing great geographical changes. In explaining the connexions between the isolated southern floras, he says, 'The most conspicuous characters that extra-tropical Africa presents in common with Australia are the abundance of the species of the following orders: Proteaceae, Compositae, Irideae, Haemodoraceae, Buettneriaceae, Polygalaeae, Restiaceae, Ericaceae, Epacrideae, Decandrous Papilionaceae, Rutaceae, Thymelaeaceae, Santalaceae, Anthospermous Rubiaceae. . . . With regard to the natural orders enumerated, their genera are almost unexceptionally different in the two countries. . . . The many bonds of affinity between the three floras—the Australian, Antarctic, and South African—indicate that these may all have been members of one great vegetation, which may once have covered as large a southern area as the European does a northern. To what portion of the globe the maximum development of this southern flora is to be assigned it is vain at present to speculate, but the geographical changes that have resulted in its dismemberment into isolated groups over the Southern Ocean must have been great indeed.'

In contrast with this, I quote one more paragraph from Thiselton-Dyer (20, p. 323): Darwin objected to "continental extensions" on geological grounds, but he also objected to Lyell that they do not "account for all the phenomena of distribution on islands, such, for example, as the absence of Acacias and Banksias in New Zealand". He agreed with de Candolle that "it is poor work putting together the merely possible means of distribution". But he also agreed that they were the only practicable door of escape from multiple origins. "If they would not work, then "every one who believes in single centres will have to admit continental extensions", and that he regarded as a mere counsel of despair, "to make continents as easily as a cook makes pancakes".

Coming now to Thiselton-Dyer's own views and those of Guppy, who has contributed probably more than any one else to the detailed study of plant migration in recent times (24, 26), we find that these authors are agreed that a theory of southward migration is the key to the interpretation of the geographical distribution of plants. Even with regard to the *Glossopteris* flora, Thiselton-Dyer says, 'I confess it would not surprise me if fresh discoveries in the distribution of the *Glossopteris* flora were to point to the possibility of its also having migrated southwards from a centre of origin in the northern hemisphere'. Guppy's earlier views were similar to those of Wallace. He sought to explain all the phenomena of distribution by migration. Later he was inclined to hesitate. As Thiselton-Dyer says, 'Guppy's heart failed him when he had to deal with the isolated case of *Agathis*, which alone seemed inexplicable by known means of transport'.

In his latest book (26), however, Guppy accepts Thiselton-Dyer's own views. He also elaborates the 'theory of differentiation', applying it first to families which, he states, tend to fall into two groups, the primitive and derivative, the first world-ranging, and the second restricted in their area. Differentiation and decrease of range go together. He goes on to apply the same principle 'in the tribe, in the genus, in the species, and in the local race or variety'. He takes the Geraniales, Geraniaceae, Geranieae, *Geranium*, sections of the genus *Geranium*, as examples, and quotes the work of Andrews on the development of the families Myrtaceae (1) and Leguminosae (2) in Australia.

Guppy further points out that, 'if the differentiation hypothesis is correct, no natural order could have been developed on the lines implied by the Darwinian theory, which, as interpreted in recent works, begins with the variety and terminates with the order, a process that reverses the usual methods of Nature'. 'Yet such a process', he says, 'as is there implied is common enough in the plant world, but it accounts not for the natural orders but for the oddities of plant forms.' 'It is here termed a specializing process in contrast with that of differentiation; but it is the differentiating process that has been the principal determining cause of diversification in plants'. There is probably much truth in this theory, though, taking into consideration the palaeontological record, it is doubtful whether it contains all the truth.

Guppy goes on to state that the differentiation theory could of itself explain distribution, if the land areas of the world were continuous and not affected by unstable climatic conditions, and he deals with the factors that modify its operation.

The theory of southward migration favoured by Thiselton-Dyer and Guppy permits of postulating the permanency of the general configuration of the land-masses of the earth's surface, and is so far in agreement with the views of Darwin and Wallace. The southern end of South Africa is a 'cul-de-sac' into which the species have poured and from which there is no escape', hence the extraordinary congestion of species there. Again, as Guppy remarks (26), 'if it can be shown, as undoubtedly the general trend of the facts of distribution does show, that the divergence of plant types responds to the divergence of land-masses from the north, and that dissimilarity is intensified with distance from that pole, any evidence for a Tertiary Antarctic centre for the flowering plants would be discounted in advance'. Thiselton-Dyer and Guppy agree that the centrifugal dispersion of species from the north, which all acknowledge took place during the last Ice period, has been repeated often during the course of geological time.

'At a time when a genial climate prevailed over the northern or land hemisphere, the plants now represented in type in the warmer latitudes occupied the regions beyond the Arctic Circle. When this period gave

4 *Bears.*—*Some General Principles of Plant Distribution as*

place to cooler conditions, the retreat to the south began; and the plants, as the diverging continents pulled them more and more asunder, became more and more distinct from each other. . . . When the warmer conditions returned, the plants advancing northwards met again in the common gathering ground around the Pole, but modified by their different experiences in southern regions lying oceans apart. There they mingled together, the eastern and the western floras, and when, with the next climatic change, they began again to retreat to their ancient home in the warmer latitudes of the south, the east had borrowed from the west, and the west from the east. The secular changes of climate have therefore tended in this way to mix together the floras of the globe.

Recently, Willis, in a series of papers (37-44), has developed what he calls the 'age and area' law, which is based on the fact that endemic species in any country tend, on the whole, to have a narrower range than non-endemic species. The older a species in any country, the wider is its range. In South Africa, where the climatic conditions are very diverse and very mixed even in comparatively small areas, the action of this 'age and area' rule is greatly modified, yet, in a general way, it seems to hold and it fits in with Guppy's theory of differentiation. 'Differentiation and decrease of range go together.' The theories of Guppy and Willis have been applied very successfully by Small (32), in his detailed monograph on the Compositae.

Most botanists since Darwin have followed him in dismissing the possibility of multiple origins. The most prominent exception is Engler, who argues in favour of polyphyletic, as he calls it (21). Drude (18) also accepted the possibility of multiple origins for major groups. Clements (15) has discussed the matter fully, and he should be consulted for further references to the literature on the subject. He says, 'The idea of polyphyletic as advanced by Engler contains two distinct concepts: (1) that a species may arise in two different places, or at two different times, from the same species, and (2) that a genus or higher group may arise at different places or times by the convergence of two or more lines of origin.' Clements accepts Engler's views and proposes that the term 'polyphyletic' should be restricted, as its meaning would indicate, to the second concept and that the term 'polygenesis' (first suggested by Huxley in the sense of polyphyletic) should be used for the first.

Clements regards the occurrence of different 'habitat forms' of the same species, e.g. of *Galium boreale* or *Aster laevis*, in different areas, but obviously related to the area of the parent form, as proof of polygenesis, and he finds further convincing evidence in de Vries's mutations. 'The evidence is conclusive that the same form may arise in nature or in cultivation, in Holland or in America, not merely once, but several or many times.' 'In the presence of such confirmation, it is unnecessary to accumulate proofs.' Clements also considers that de Vries's work proves the

possibility, not merely of the polyphyletic of genera and higher groups, which, since the appearance of Engler's work, has been very generally accepted by botanists, but also for species. 'De Vries found that *Oenothera nanella* arose from *O. lamarckiana*, *O. laevifolia*, and *O. scintillans*; *O. scintillans* arose from *O. lata* and *O. lamarckiana*; *O. rubrinervis* from *O. lamarckiana*, *O. laevifolia*, *O. lata*, *O. oblonga*, *O. nanella*, and *O. scintillans*, &c.' Numerous similar examples could be applied from the recent literature of genetics.

If the polygenesis of species, particularly, is to be accepted as an established fact, the difficulties of explaining the distribution of many poly-genetic species at once disappears; but, of course, there has been no intention on the part of any one of asserting that all species originate in this way. It will be shown later that in the case of certain South African endemics a polygenetic origin is at least extremely probable.

Schönland (29) has summarized some of the facts and theories concerning the origin of the angiospermous flora of South Africa, and he remarks at the outset that 'the question of the origin of our flora having been approached almost entirely by European botanists, who were naturally influenced by the leading features of the development of the floras of the northern hemisphere, it has sometimes greatly suffered through forced interpretation of facts and supposed facts (compare, e.g., Thiselton-Dyer, 1878)' (19). Schönland contends that the data for an adequate treatment of the subject are absent even to this day, and that there is need for caution in dealing with the whole question. Nevertheless, though he kept in mind Wallace's warning that it is 'so easy and pleasant to speculate on former changes of land and sea with which to cut the Gordian knot offered by anomalies of distribution', Schönland, after considering the facts of distribution in a number of separate families, found himself forced to the conclusion that in late Mesozoic times, possibly even up to the Cretaceous period, there was still a direct land connexion between Australia and South Africa and, possibly even in Tertiary times, between tropical Africa and America.

Recently Schönland (30) has given a summary of the distribution of the genera of South African flowering plants with special reference to those found in the divisions of Uitenhage and Port Elizabeth without discussing further the question of origins.

The axiom that the present is the key to the past has been accepted ever since the time of Lyell, at least theoretically, but biologists have not always been willing to use it as much as they should in practice. Not only should it be applied to geology and biology, but also to climatology. Until we know as much as can be known about present-day tendencies, it is well to be cautious about dealing with the past, but I agree with Schönland that, so far, all the evidence goes to show that the climate of South Africa,

6 *Bews.—Some General Principles of Plant Distribution as*

though it is subject to extreme fluctuations, has not altered, materially at least, since Upper Cretaceous times. I have pointed out elsewhere how tree-veld in South Africa has not, as has often been supposed, replaced more mesophytic forest (9), and how our extensive grasslands, though they often give way to forests (11), yet must have existed for a very long period of time, since most of our mammals in South Africa are adapted to grassland conditions (7, p. 150). In a word, present-day conditions, in a general way, as regards the main types of vegetation, must have existed for a very long period of time.

It is the object of the present paper to consider chiefly present-day tendencies in plant distribution in South Africa. The fact that these often do throw light on the past will be treated incidentally. Attention, therefore, is directed chiefly to the distribution and origin of species rather than to higher groups, a method which differs from that pursued by most plant geographers.

PRESENT-DAY CONDITIONS IN SOUTH AFRICA.

It is, of course, impossible, within the limits of the present paper, to give very full details regarding the distribution of the various types of vegetation in South Africa, nor is it necessary for our present purpose. It will be sufficient to recall the leading facts. Details have been given by the writer in various papers (4-13). There are many distinct climatic areas in South Africa, and there is a distinct advantage to be gained by arranging them in a series, beginning with the most adverse types and ending with the most favourable. Any great subdivision will not be attempted here, although, if small differences were taken into account, the series could be made a very long one. Even if space permitted, it is doubtful whether great subdivision is desirable, for the study of experimental plant ecology has shown that environmental differences as regards water content, humidity, light, or temperature have to be considerable to cause any distinguishable morphological adjustment in plants. They are, as Clements (15) has shown, much greater than the unit differences recorded by instruments. 'In short, the differences of habitats as ascertained by thermograph, psychrometer, and photometer, are much greater than their efficient differences, and, with respect to their ability to produce modification, habitats fall into relatively few categories.' In South Africa, too, many species are so adaptable as to be able to inhabit rather widely different habitats, e.g. two or more of those given below. Though differences in soil conditions are efficient in causing changes, yet in South Africa the climatic factors are of more importance. The chief climatic areas, roughly arranged in order of increasing mesophytism, are the following:

(1) *Western region.* The Namib and stony or sandy desert areas of the western side. The rainfall is very irregular and small in amount.

What does fall is usually summer rainfall, but whole years may pass without any. The average temperatures are lower than at corresponding latitudes on the eastern side, owing to the absence of the warming influence of the Mozambique current. The region becomes moister and more tropical towards the north and east. The soil is mostly sandy or stony. Vegetation of scattered xerophytes in the driest parts, passing first into *Aristida-veld* and then into more mesophytic grass-veld and tree-veld in the moister eastern and northern portions.

2. *Central Karroo region.* The rainfall is small in amount (3-14 inches) but more regular, increasing in amount from west to east. General altitude from 1,800 to 2,500 ft., but the mountains which form the escarpment of the central plateau are from 4,000 to 8,000 ft. high, running from west to east. These have many species belonging to south-western or eastern genera. The temperature of the Karroo shows extremes of heat and cold, with frosts in winter. It forms a climatic barrier of considerable importance between south-west and east, and it would be still more important were it not for the mountains that cross it. The Karroo soils are hard-baked clay, very rich in salts. The vegetation is composed of dwarf shrubs and succulents.

3. *Cape or South-Western region.* Winter rains and dry, hot summers. Annual rainfall 20 to 40 inches. In places the rainfall amounts to 60 inches or more and the summer heat and drought is tempered by south-eastern mist-clouds. Temperatures, except during the summer months, fairly low, but actual frosts, except at high altitudes, are not common. Soils varied. Dominant vegetation of sclerophyllous shrubs with patches of eastern forest in moister situation. Flora shows affinities with South-West Australia, and also, to some extent, with the Mediterranean and Europe, though the species in nearly all cases are distinct.

4. *Sand-veld Region of the Kalahari.* Summer rainfall increasing from about 10 to 20 inches from west to east. Temperature considerably higher, becoming tropical towards the north. Sandy soil or sand-dunes with underground drainage. No surface water. Vegetation of grass-veld passing into tree-veld. Flora with close eastern or tropical affinities.

5. *Dry Thorn-veld (Low-veld) and Succulent Scrub Areas of the Eastern side.* Summer rainfall 20 to 30 inches. Great extremes of temperature. Very hot in summer, regular frosts in winter. Hot winds an important feature. Soils dry-baked clays, rich in salts (cf. the Karroo, which these areas resemble in many respects). Vegetation of grass-veld, tree-veld, succulent and thorny scrub.

6. *High-veld and Mountain Areas of the Eastern side.* Summer rainfall 30 to 50 inches and also mist-clouds. Temperature range (both daily and yearly) not so great as in the low-veld of the valleys, but frosts in winter are experienced outside the forests. Soils loose, deep, well-

8 Bews.—Some General Principles of Plant Distribution as

aerated loams, or sometimes sandy, poor in plant food. Vegetation grass-veld, scrub, and forest.

7. *Coast-belt of the Eastern side.* Summer rainfall 30 to 40 inches. Temperatures uniformly fairly high, but not extreme. Frosts absent. Soils varied. Vegetation grass-veld, scrub, and subtropical forest. Flora with close tropical affinities (see Bews, 12).

WIDESPREAD SPECIES.

Species which belong to the climax plant communities in the western, south-western, and central regions often are found to belong also to early stages of the plant succession on the eastern side, so that the arranging of the climatic areas in an ascending series reproduces, to a certain extent, the various stages of plant succession in the higher types. Any species which, under favourable conditions, is able to oust another species is to be reckoned of a higher ecological type. The primitive ecological species tend to be widespread, the higher types more restricted in their ranges. For instance, if we consider the 320 species which are common to the Cape Peninsula and Natal, we find that, while they belong to widely different growth-forms and show no possible phylogenetic relationships, they practically all agree in belonging to early stages of the succession as ruderals in subseres or marsh and aquatic plants in the hydrosere, as littoral plants, or as xerophytic light-demanding species in the xerosere (13).

A little consideration will show why, in a country like S. Africa, widespread species usually belong to early stages of the succession. We have seen that South Africa shows a number of distinct climatic areas with increasing mesophytism in a general way from west to east, but with a south-western area, which, though it has a fairly high rainfall, receives that rainfall in winter instead of summer and is separated from the regions of summer rainfall by the dry Karroo. Nevertheless, traversing all the areas there are found primitive habitats, which are to a large extent uniform. They are:

1. Cultivated land, waste places, &c., with ruderal species.
2. Lakes, streams, marshes, with aquatic or semi-aquatic species.
3. The seashore habitat, mostly sandy, with widespread littoral or sand-dune plants.
4. To a less extent the mountain ranges which cross South Africa.
5. Drier areas in the (thorn-veld) river valleys, open rocky situations on steep slopes, &c., are common all over the eastern side and differ but little from the Karroo and western areas as a whole. They are occupied by primitive ecological types which are soon ousted by others in the east.

In addition to these primitive habitats, it should be remembered that South Africa lies south of a fairly uniform tropical region to the north.

where tropical species are able to cross the continent without encountering any barriers. This explains the connexions between the subtropical areas in the west and those in the east, but not the connexion between the Cape Peninsula and Natal.

THE ORIGIN OF NEW SPECIES AND VARIETIES.

Species which occupy any of these primitive or relatively primitive habitats listed above naturally have no difficulty in spreading all over South Africa, and a large number of them are thus widespread. Now, if a species which is adapted to extreme conditions in the course of its spread comes into contact with more mesophytic conditions it is, as a rule, rapidly ousted in the course of plant succession by more vigorous growing, more mesophytic species. But if the primitive species is capable of varying, it may give rise to a new variety or it may produce a new species capable of holding its own with other competitors. Many examples of this will be given later. The genus *Klus*, e.g., has many species which have developed in this way.

It is a well-known fact among gardeners that the cultivation of a species often leads to variation or mutations. The same thing would appear to happen in nature when a species in the course of its migration comes into environmental conditions which differ from those which produced it. In South Africa this, as a rule, implies a change from xerophytic or hydrophytic to mesophytic conditions, since the former are widespread, the latter more local. That is why widespread species belong to early stages of the succession. Succession is usually towards the mesophytic. If, however, mesophytic conditions were widespread and xerophytic conditions local, the reverse might happen. A widespread mesophytic species in the course of its wanderings might give rise to one more xerophytic. This is of much less importance in South Africa at the present time, whatever it may have been in the past. However, another type is important, namely, when a tropical species which cannot withstand frosts gives rise to a temperate species which can. It has already been pointed out that to the north of South Africa tropical species are able to spread across the continent. Derived subtropical or warm temperate species invade South Africa at different points.

VARIOUS METHODS OF ORIGIN.

When we seek to apply the process just outlined to concrete examples, we find that these tend to fall into several different categories, and the process itself requires further analysis. If for 'species' we read 'varieties' in each case, the number of possible examples is, of course, enormously increased.

1. A species A, which is widely distributed, may give rise to a closely allied but much rarer, more mesophytic species B in Natal, another closely

allied species C in the northern Transvaal, and still another D, more xerophytic perhaps, in Namaqualand, &c. This is a very common type of occurrence, and in some of the larger genera numerous rare endemics can be grouped around a single widespread species in this way. Even in smaller and more uniform areas, such as the south-western region or Natal, the same thing can be shown to take place. In other cases, only two species are involved, the one widespread, the other usually more mesophytic and always more restricted in its range.

2. A species A is produced in or brought into a certain suitable climatic area and it spreads, until it reaches the limits of that area. Then it gives rise to another closely allied species B, which spreads over the next climatic area, and the process may even be repeated with the production of a species C. Examples of this can be given, but it is hardly worth while speculating whether B could again produce A on the other side of B's own area. As long as one is only dealing with habitat varieties, no doubt this could happen, but with differences of specific rank the matter would be considered highly controversial and it would be impossible to prove in any particular case that A had not been distributed to the other side of B's area by ordinary methods of migration.

3. Several species A, B, C, &c., which are closely allied are often found to have more or less the same distribution. Different explanations of their origin are possible. Species A may have given rise to B and to C; or A may have given rise to B and then B to C; or, on the other hand, an ancestral species may have broken up into A, B, and C.

4. The same kind of thing may happen in the case of various species A, B, C, &c., which do not have the same distribution but whose areas overlap, though this category is hardly worth distinguishing.

5. *Polygenesis*. There are several examples to be given later of a widespread species A, and another closely allied species B, the latter having widely discontinuous distribution. B is often a subtropical species recorded only for the Natal coast-belt and the northern Transvaal, or for the north-western and again for the north-eastern side, while A is a tropical species widespread to the north. Or A may be a widespread species in one of the primitive habitats from Natal across the Karoo to the Cape, while B occurs only in Natal and at the Cape in mesophytic forest habitats. Now the usual assumption has been that B has migrated from one mesophytic habitat to the other, or from one subtropical habitat to the other, an assumption which of course it is difficult to disprove and probably is correct in many or indeed in the majority of the cases. Ordinary migration, of course, must explain things when B, as in the case of many forest trees, is an isolated species without any closely connected species A growing in between its mesophytic habitats. It is worth noting that, in such cases, the fruits or seeds are usually distributed

by birds. In other cases, where there is present the widely distributed and closely related species, polygenesis would appear to afford a much simpler explanation. It would appear, therefore, that we must admit at least the possibility of polygenesis, for, if it affords a simpler explanation than ordinary migration over enormous areas, then the onus of proof is on the side of those who deny the possibility of multiple origins. Personally, I would go farther and advocate its extreme probability. As we have seen, Clements regards it as definitely proved by the work of de Vries.

If now we extend the facts learned about the species to the genus, tribe, and family, we may take it for granted that the same methods will apply. We find widespread families like the Scrophulariaceae have given rise to families with a restricted distribution like the Selaginaceae, which is mostly S. African, with a few isolated genera to the north, or the Myoporaceae, which are mostly Australian, with isolated examples elsewhere as far apart as China and the West Indies, and one genus *Oftia* in S. Africa. Some authorities sink these families as tribes of the Scrophulariaceae, but this does not affect our argument. Again, in the Verbenaceae, Iridaceae, and other families we find the oldest tribes or sections of the family concentrated in the south-western region of South Africa, while other younger tribes or sections are eastern. All these facts are very significant. Instead of beginning with the whole world's flora and finding connexions between the Cape and Australia, for instance, we begin with a restricted area and study the origin of species. Then with caution our methods and observations may be extended to a wider area and the time lengthened from the present into the past, always keeping in mind that the only key to the past is to be found in the present.

THE EVIDENCE.

It may be well to anticipate one or two obvious objections which may be brought forward to accepting certain of the following examples as evidence supporting the above conclusions. The first is that our knowledge of detailed distribution in South Africa is still far from complete. Species which are now thought to be restricted or discontinuous in their distribution may very well subsequently be shown not to be so. This objection is valid, no doubt, in some cases, but it certainly cannot apply to them all. Its weight as an argument is in inverse proportion to the number of examples given.

The second objection is that the distribution in each example given can be explained by ordinary migration and its abundance or rarity by adaptation to environmental conditions. As regards the first part of the objection, it is, as already admitted, difficult to disprove in any particular case, nor do I seek to deny that it is usually a sufficient explanation, especially in the case of species distributed by birds. As regards the second part of the

objection, it would, in this case, be brought forward only to justify the first, since some explanation on the theory of migration would naturally be expected of one species being widespread and another closely allied species having restricted or discontinuous distribution. The objection as a whole applies chiefly to polygenesis as an explanation of discontinuous distribution. The case for polygenesis has already been sufficiently argued, and it is maintained that the onus of proof is on the other side. It is clearly as difficult to disprove polygenesis as to disprove migration, and the former, in many cases, is the simpler explanation. Some critics would admit that widespread species might produce closely allied but not identical species at two or more separate points, i.e. they readily admit No. 1 but deny No. 5 of our categories as given above. In reply to this, it might be asked, how often does it not happen that the systematist allows himself to be influenced by geographical distribution in deciding that two closely allied species are distinct? It makes him magnify minor differences which are not constant and it makes him sometimes see differences which do not exist. The result is clearly reasoning in a circle. Species are said to be distinct simply because they are widely separated geographically and the chances of migration are remote, and then it is denied that identical species can arise in separate geographical areas.

A third objection is that many of the examples quoted are not really distinct species, though they are so described in the 'Flora Capensis'. That probably in many cases is quite true, but it is not really an objection, for, though they may be considered merely varieties, the same evolutionary principles are involved. In fact, it has only been because it was thought unnecessary that the words 'or variety' were not repeated after 'species' in each case above. The 'Flora Capensis', too, has been the work of many different specialists, and it is unlikely that they all have erred in the making of too many species. The earlier work of Harvey more probably erred in the other direction, for he was not inclined to split species too much. In a large flora of about 13,620 species the mass of evidence to be sifted is so great that the presentation of it is a matter of considerable difficulty. Considerations of space prevent us from dealing with all the families. An effort has been made to make as varied a selection of examples as possible, and if any reader is still not convinced, he can read through the 'Flora Capensis' for himself and find many more. Our aim for the moment is to illustrate the method, and its bearing on other aspects of botanical study will be discussed later. Though the 'Flora Capensis' has been mentioned, it must be admitted that the earlier volumes are so incomplete as a record as to be of little use, though the later volumes are better. I have relied chiefly for details regarding distribution on the various local check-lists supplemented in the case of Natal by my own various field investigations. The lists referred to are those by Bolus and Wolley-Dod for the Cape (14).

Medley Wood for Natal (45), Phillips for Basutoland (28), Eyles for Rhodesia (23), and Burt Davy and Pott-Leendertz for the Transvaal (17), though the last mentioned does not give any localities or any information regarding relative abundance. Since most of the check-lists and the 'Flora Capensis' adhere to Bentham and Hooker's arrangement of the families, we shall also arrange those selected in the same order.

Ranunculaceae.

The genus *Clematis* is widely distributed through temperate countries, occurring in the tropics chiefly in mountainous regions. *Clematis Thunbergii* extends from Abyssinia to South Africa. *C. brachiata*, which is closely allied, is more distinctly South African, extending from the Cape to Natal and Rhodesia, but not farther north. This illustrates a species extending over one area and being continued in another area by a closely allied species. Other species of the genus differ in minor characters, and their distribution illustrates our principles extremely well. *C. Owenii* differs from *C. brachiata* in its filaments and anthers. It is a rare species with discontinuous distribution at Inanda (Natal) and again in Zululand. Then again we have *C. glaucescens* in the Tugela district, Natal. *C. virgata* in southern Rhodesia, *C. Wrightiana* at Chirinda, all allied to *C. brachiata* and all rare endemics, so that within the limits of this single genus we find examples of most of our above-named categories. Oliver, in 'The Flora of Tropical Africa', mentions other interesting points. *C. chrysocarpa* is recorded from the Nile and again from Angola on the west side, and is very closely allied to *C. trifida*, a Madagascar plant. *C. Kirkii*, Mozambique, is nearly related to *C. Roperi*, Madagascar. *C. Stanleyi*, a suberect shrub like *C. Kirkii* and *C. chrysocarpa*, is found in Angola and again in the Transvaal, but the record for Natal is doubtful. Some of the species of *Ranunculus*, being water or marsh plants, have spread all over South Africa. Other species of *Ranunculus* are more or less rare endemics, e.g. *R. capensis* at the Cape, *R. Meyerii*, King William's Town, Natal, and the Transvaal at isolated points, *R. Faurii* and *R. Copei* on the Drakensberg. *Anemone capensis* is confined to the south-western region. *A. caffra*, which belongs to the same section (*Pulsatilloides*) of the genus and is fairly closely related, occurs only on the eastern side. *A. Faurii*, which again is allied to *A. caffra*, is a rare Drakensberg species, and three other species are recorded for the Transvaal.

Thalictrum rhynchocarpum is one of the most interesting species in the genus, having only a single carpel. It occurs from Natal to Abyssinia at the edges of bush and in sheltered places. It has no close ally, though the north temperate *T. minus* also occurs in South Africa. The genus *Koeberlinia* is exclusively South African. *K. vesicatoria* is the widespread species from the Cape to Natal. At the one end of its area, the Cape, it has *K. rigida*, *K. gracilis*, *K. hirsuta*, and *K. duncifolia* related to it; at the

14 Bews.—Some General Principles of Plant Distribution as

other end of its area, in Natal, it has *K. brevistylis* (recorded only for Inanda and for Zululand). The Ranunculaceae are a small family in South Africa, and to deal with other larger families in the same detail would be tedious. If many families are omitted altogether and others are dealt with very briefly, it is not because they do not provide equally good examples.

Cruciferae.

The genus *Heliophila* is endemic in South Africa. It is characterized by a peculiar embryo, the cotyledons being twice folded transversely. There is one species in Rhodesia, two in the Transvaal, four in Natal, and between fifty and sixty at the Cape. Excellent examples of our general principles may be found among the species concentrated at the Cape, showing that they apply to small areas as well as large. Thus *Heliophila diffusa* is marked by Bolus and Wolley-Dod as 'frequent' in the Cape Peninsula: *H. peltaria*, 'in similar situations but rarer', we find differs only in having shorter pods and two or three seeds instead of four to eight: *H. pilosa* is common all over the south-western region. Several other closely allied species, *H. graminea*, *H. linearis*, *H. stricta*, *H. divaricata*, are much rarer. Similar relationships are found among the shrubby species.

Bixaceae.

The genus *Kiggelaria* affords one of the best examples of a widespread xerophytic light-demanding pioneer shrubby type giving rise to a mesophytic forest tree type or possibly vice versa. Harvey, in the 'Flora Capensis', allows three species to stand—*K. africana* and *K. ferruginea*, both xerophytic; and *K. dregeana*, the forest type. Sim, in his 'Forest Flora' (31), sinks them all in one, *K. africana*, but admits that the forms are distinct. Such differences of opinion regarding specific rank are, of course, always bound to arise. The distribution of the forest forms is widely discontinuous, like the forests themselves.

Polygalaceae.

Several species of the large genus *Polygala* illustrate at the present day in South Africa the process of breaking up. *Polygala oppositifolia* is common from the Cape to Natal and Delagoa Bay. It is a typical pioneer of scrub occurring around the margins of bush. It has given rise to many more mesophytic forms. Harvey recognized eight varieties, but most of these, as well as others, have been recognized as distinct species by various authors. *P. myrtifolia* is another widespread species, which is very variable in its floral characters and leaves. In this case, Harvey recognized four varieties, all elevated to the rank of species by other authors. The fact that gradations between the distinct varieties can be recognized does not of course necessarily force them to fall into one species, for in a type like this hybridizing is quite likely.

P. virgata is also widespread and has five varieties, according to Harvey, and in addition a number of allied species, with more restricted distribu-

tion. *P. bracteolata* at the Cape has, according to Bolus and Wolley-Dod, produced a variety *umbellata* adapted to higher elevations on the hillsides. The genus *Mundtia* has two species, one at the Cape, the other in Brazil. *M. spinosa* has three varieties. *Muraltia* is a South African genus with about fifty species in the south-western region, four in Natal, two in the Transvaal; cf. *Heliophila*. *Securidaca*, on the other hand, is a typical genus with no representatives farther south than Rhodesia and the Transvaal. One species, *S. longipedunculata*, is very widespread from Abyssinia to the Transvaal and is very variable. The genus is much better represented in tropical America.

Malvaceae.

This tropical or subtropical family has many representatives which have spread over South Africa as over other temperate regions in other parts of the world. Species of *Sida* and *Abutilon*, genera well represented in the tropics, spread as weeds mostly over the eastern side. *Pavonia* is chiefly American, but some of the tropical African species (e.g. *P. macrophylla*, *P. odorata*) are very widely distributed from Natal to Abyssinia, and even to India. On the other hand, there are several endemics with narrow ranges (e.g. *P. Dregei*, *P. Kraussiana*) around the margins of coast-belt bush. *Hibiscus* as a genus is distributed all over South Africa and several of the species are interesting. *H. tiliaceus* is a tropical or subtropical sea-shore species, very distinct and remaining so widely distributed all round the tropics. *H. trionum* is a very common subtropical weed, which gives rise to numerous distinct varieties. *H. aethiopicus* occurs all over South Africa and, like many others, could be broken up into several distinct species. *Malvastrum* is a genus with some twenty species mostly concentrated in the south-west, only one, *M. capense*, reaching Natal. It also comprises many American species.

It will be seen that, in the course of our survey of the families, we meet with many examples of isolated species with no obvious connexions, whose distribution can only be explained by ordinary methods of migration, e.g. *Hibiscus tiliaceus*. It is not our immediate object to give examples of these, except incidentally, but they will be referred to as a class later.

Sterculiaceae.

The genus *Hermannia* (including *Mahernia*) is a very large one, distributed over all South Africa, and with over 100 species. Again it illustrates most of our points. We find variable and widespread species like *H. candicans*, *H. salvifolia*, *H. flammea*, in the process of breaking up into several distinct varieties or species; we find *H. cuneifolia*, which is common at the Cape, replaced on the dry plains around Graaff Reinet by the nearly related *H. desertorum*; we also find that at the Cape itself *H. cuneifolia* has given rise to two other closely allied but rarer species, *H. decumbens* and *H. alnifolia*. The eastern species, in most large

genera distributed over South Africa, tend to fall into a group by themselves, distinct from the south-western. The genus *Dombeya* is tropical in its affinities. The South African species all come close together. *D. natalensis* and *D. rotundifolia* are both common in the coast and mid-lands of Natal (the latter also in Rhodesia). *D. dregeana*, which extends from Natal to Uitenhage, only differs in the flower-buds, leaves, and involucre leaflets from *D. natalensis*. To the north, in the Transvaal, there are six other species, and the genus is still better represented in the tropics. We thus see, as in so many other cases, the various steps of invasion from the tropics into South Africa, with consequent modification.

While the genera *Hermannia* and *Dombeya* afford perfect examples of the working of our general principles, when we turn to the genus *Sterculia*, as represented in South Africa, we find a case of the most extraordinary isolation. The genus is most abundant in tropical Asia, but there are seven species in the 'Flora of Tropical Africa' and one, *S. murex*, in the Lydenburg district of the Transvaal. *S. Alexandri* (Harv.), however, occurs completely isolated near Uitenhage. Harvey says it is allied to *S. foetida*, but differs in various characters, and adds, 'No one seems to have met with it but Dr. Alexander Prior, who found but a solitary tree in a narrow kloof, somewhere among the Van Staadem Mts., a locality rich in interesting plants and probably still concealing other novelties.' Schönland (30) refers to its complete isolation, but he does not say whether it has been collected since.

Tiliaceae.

The genus *Grewia* is a great tropical African genus which has produced species capable of invading South Africa. *Grewia occidentalis* extends from Abyssinia to the Cape Peninsula. It is a typical pioneer in the xerosere, important in establishing scrub and forest. *G. caffra*, which is common in the Natal coast-belt bush, is probably derived from it though specifically distinct, and many of the other rare species might also be connected. No better example could be found of the first of our categories, but cases of discontinuous distribution would be discounted as examples of polygenesis, because of the edible drupes. The genus *Sparmannia* has *S. abyssinica* not known outside Abyssinia, but apparently intermediate between the two South African species *S. africana* and *S. palmata*.

Geraniaceae.

Pelargonium is a large and characteristic Cape genus with a few outliers in tropical Africa, western Asia, and Australasia. We find that *P. multibracteatum* from Abyssinia is closely allied to *P. alchemilloides* from the Transvaal Natal, and the Cape, the latter being a variable species. *P. flabellifolium* is common in the upper districts of Natal and is also recorded for Angola and the Transvaal. There are seventeen species altogether in Natal and ten in the Transvaal, but the largest section of the genus, the section *Hoarcea*, is altogether south-western. Many of the species

are closely allied and some single species are extremely variable, e.g. *P. myrrhifolium*, of which Harvey gives nine varieties that have been described as distinct species by various authors. Though the eastern species are, as a rule, distinct from the south-western, in some cases they are nearly related, e.g. *P. lobatum* and *P. pulverulentum*. Only one or two, e.g. *P. alchemilloides*, *P. capitatum*, are common to Natal and the Cape. *P. inquinans*, the parent of most of the 'scarlet geraniums', extends from Natal to Uitenhage. The general distribution of the genus, with its fairly numerous outlying species and greatest concentration at the Cape, is the chief point of interest, since it is paralleled in so many others and throws light on the question of the origin of the south-western flora.

Oxalidaceae.

The only genus is *Oxalis*, with more than a hundred species, again chiefly concentrated at the Cape, but with seven in Natal and seven in the Transvaal.

O. corniculata is a ruderal, common in subtropical regions. The Abyssinian species *O. obliquifolia* is very near the Cape *O. convexula*, and *O. caprina*, another Abyssinian species, is also found at the Cape. The genus illustrates generally the same features as *Pelargonium*.

Rutaceae.

Calodendron capense, the Cape Chestnut, is a monotypic species, a large forest-tree whose distribution is to be explained only by migration. It is found in all the eastern forests and has no near allies. *Xanthoxylon capense* (Fagara), on the other hand, illustrates exactly the same points as *Kiggelaria* (Bixaceae) described above. Sim (31) sinks all the forms in one species, but the xerophytic forms are widespread, the more mesophytic forms have restricted and discontinuous distribution. The genus is tropical. *Toddalia lanceolata* (eight stamens) is widespread from the Cape to Mozambique and Mauritius. *Toddalia natalensis*, four stamens, but otherwise very similar, is only found in the Eastern Province and Natal. *Clausena inaequalis* is a Natal and tropical species, allied to the Indian *C. Willdenowii*.

The Rutaceae of the south-western region are strongly scented xerophytic shrubs, very different in every way from the eastern forest species. There are nearly two hundred species in the south-west, belonging to nine genera, of which *Agathosma* includes about half. Only one species, *Barosma lanceolata*, reaches Natal. This marked contrast and differentiation between east and west in the family is very striking.

Celastrineae.

The genus *Celastrus* illustrates again our chief points. The tropical African species appear to be all endemic, with mostly narrow ranges, except *C. senegalensis*, which extends from Mozambique to the Mediterranean and eastward to India. The twenty odd South African species are all different.

C. buxifolius is a widespread rather variable pioneer species all over South Africa, and is of considerable importance in the establishing of thorn-veld and scrub, though some of its more mesophytic varieties occur in the forest. *C. peduncularis*, which is without spines, is a large forest-tree common in eastern forests. *C. acuminatus*, like *C. buxifolius*, is widespread, usually outside but sometimes inside forest, and several other species behave in the same way. Some of the species have become adapted to coast sand-dune conditions, e.g. *C. maritimus*, or coast forests, e.g. *C. cordatus*. According to Sim's information regarding distribution (31), *C. undatus* in forest and scrub would appear to take the place of the pioneer *C. alatus*, which is closely allied (31, p. 186) and occurs on exposed rocky krantzes.

The relationship between the species of *Elaeodendron* is exactly the same as in the case of *Celastrus* and other large genera of trees and shrubs. A number of species are light-demanding pioneers, low-growing and shrubby, while others, like *E. croceum* (Saffron Wood) or *E. sphaerophyllum*, are large mesophytic forest-trees.

Ampelideae.

The numerous species of *Vitis* (*Cissus*) are nearly all eastern, *V. capensis* being the only species which reaches the Cape Peninsula, though several others reach the eastern parts of the south-western region. The headquarters of the genus is, of course, in the tropics, and again various steps of invasion into South Africa can be studied, as can be done also with members of the family Sapindaceae; but a still better field is afforded by the next example.

Anacardiaceae.

The genus *Rhus* illustrates better our various principles than probably any other genus, and it deserves further study from this as well as other standpoints. Not only is the genus very well represented in South Africa, but it extends through tropical Africa, North Africa, the Mediterranean, Arabia, India, the Himalayas, China, North America, Mexico. Schönland (29) says about it, 'No less than eleven species occur on the Cape Peninsula alone, yet the close analysis to which the sect. *Gerontogae*, Engl., which includes our own species of *Rhus*, has been subjected by Diels, leads him, and I think rightly to the conclusion that this section has branched from the type (*stamm*) of the genus, presumably during early Tertiary in the southern portion of the eastern part of the northern hemisphere. Originally it probably included forms the organization of which was adapted to moderately dry and sunny localities. When, in the course of the Neogene, the geological revolutions in western Asia and Europe caused migrations on a large scale, and at the same time more intimate relations were established between East Africa and the Indian region, *Rhus* took part in the general invasion of Eurasian types into Africa and there commenced in numerous regions a more diverse development than in its original home.'

When we look at the present-day distribution of the species of *Rhus* in South Africa, we find certain of them (e.g. *Rh. dentata*, *Rh. villosa*) very widespread, as pioneer species in the xerosere. Others (e.g. *Rhus obovata*) occur along the streams and are important in the hydrosere, or (e.g. *Rh. crenata*) occur on the coast sand-dunes in the psammosere. As succession advances, we find species of *Rhus* (*Rh. dregeana*, *Rh. erosa*) dominant in climax vegetation on the dry doleritic Karroo kopjes. These two species are closely allied and may have been derived one from the other or by the splitting of an ancestral type. In more mesophytic forest areas we find various steps in the adaptation to more favourable conditions in a large series of forms till we reach such large forest-trees as the Red Currant (*Rh. laevigata*), which grows fifty to eighty feet high, and two to four feet in stem diameter. *Rhus* also shows adaptation to purely grassland conditions in the species *Rh. discolor*, which takes its place with numerous other associated plants of the grassveld. There are few examples of adaptation to grass-land conditions from the genera of trees and shrubs, the evolution of species being, as far as South Africa is concerned, usually in the other direction, widespread xerophytic types producing more mesophytic forms.

Leguminosae.

It will be necessary now to pass over certain large and important families in the briefest possible way. The present-day development of the Leguminosae could be made the subject of a monograph by itself. If we take any two allied species or any group of allied species, again and again we find that species A with a wide distribution has apparently produced one or more, B, C, D, &c., each with restricted and in some cases with discontinuous distribution, or there has been an apparent breaking up. Examples are seen in the genera *Podalyria*, *Crotalaria*, *Rafnia*, *Lotononis*, *Argyrolobium*, *Lebeckia*, *Buchenroedera*, *Aspalathus*, *Psoralea*, *Indigofera*, *Tephrosia*, *Lessertia*, *Vigna*, *Dolichos*, *Eriosema*, and *Acacia*. At the same time examples of isolated species with no obvious connexions are to be found, e.g. *Sutherlandia frutescens*, but they are not numerous. The suborders should, of course, be studied separately. The Caesalpinae invade South Africa from the tropics only along the eastern side as far as Uitenhage, and in the case of *Schotia speciosa* as far as Mossel Bay. Several of these tropical outliers in South Africa are interesting, e.g. *Bauhinia tomentosa* extends from Mozambique to Natal and is recorded from Angola. In South Africa, *B. garipensis* in Namaqualand. *B. Bowkeri* 'along the Bashe River, Kafraria', are both closely allied.

The Mimoseae has the great genus *Acacia*, which is all over Africa and is even more characteristic of Australia. *Acacia arabica* extends from India through the whole of Africa to Natal, though the Natal variety *Kraussiana* is now separated as a distinct but allied species, *A. Benthami*.

A. horrida (*A. Karroo*) is commonest and most widespread in South Africa, being the most important pioneer tree in dry areas. It is interesting to notice its adaptation to more mesophytic conditions on the Natal coast-belt. Some species of *Acacia*, e.g. *A. Kraussiana*, *A. pennata*, have adopted the climbing habit in the bush. *A. caffra* has become adapted to moister conditions near stream-banks. Like so many of our larger genera, *Acacia* gives excellent opportunities of observing adaptation to various ecological conditions, but the majority are extremely xerophytic thorn-veld species. The Papilionaceae fall into two fairly well-marked distributional groups, an eastern and a south-western with eastern outliers in the south-west, e.g. *Crotalaria* and *Vigna*, and south-western outliers in the east, e.g. *Podalyria*, *Lebeckia*, *Rafnia*, but several large genera are distributed all over. The subject is too big to discuss in detail here.

I must pass over, for the same reason, such large genera as *Crassula* and *Mesembrianthemum*, though specialists dealing with them could illustrate many interesting points with regard to their distribution.

Rubiaceae.

This is one of the largest families of flowering plants, and is chiefly tropical and subtropical in both the Old World and the New. It is particularly interesting to follow the steps of its invasion into S. Africa, where it has produced not only large numbers of endemic species, but even characteristic endemic genera, e.g. the three belonging to the tribe Anthospermeae, viz. *Galopina*, *Nenax* (*Ambraria*), and *Carpococ*, with 2 to 4 species each, *Galopina*, from Natal to Swellendam, *Nenax*, East London to Capetown, and *Carpococ*, Grahamstown to Capetown. The centre of distribution of these anthospermous Rubiaceae has been, of course, thought to be rather from the south-west than towards it (27, 29). Another endemic genus is *Burchellia* (*B. capensis*), which is common from Natal to the Cape, but is certainly eastern or tropical in its affinities and origin, belonging as it does to the tribe Gardenieae. The distinctly herbaceous genera, e.g. *Galium*, *Anthospermum*, *Oldenlandia* (*Hedyotis*), are generally distributed over S. Africa with others (*Pentanisia*, *Spermacoc*, *Hydrophyllax*, a strand plant) not quite so far to the south-west. *Pentanisia variabilis*, as its name implies, is extremely variable, and would repay intensive study from the distributional standpoint.

The shrubs and trees are mostly eastern, being very common around the margins of the forest and in scrub in Natal, especially on the coast-belt (see 12). The herbaceous genera are very distinct in every way from the shrubs and trees. Among the latter, the differences one notices as one passes from the subtropical Natal coast to the Midlands are very striking. Thus *Randia dumetorum* is a species which extends all through the tropics from Hongkong and India to Durban. Another species, *R. rudis*, is very common in the midlands of Natal, but is not tropical. It extends as far as

Grahamstown, and is one of the first arrivals under the pioneer thorn-trees in the thorn-veld (9). *R. parvifolia* (listed by Medley Wood) is hardly distinct from *R. rudis*. Similar relationships may be observed between *Gardenia Thunbergia*, a very widespread tropical species, and other South African species of *Thunbergia* (cf. Stapf and Hutchinson, 34). The genera *Trigalysia*, *Plectronia*, *Vangueria*, and *Pavetta* also illustrate our general principles.

Pavetta caffra is chiefly a coast-belt species. *P. Cooperi* is a very closely allied species, and is only found in the midlands. The genus *Plectronia* extends from Natal to the Cape Peninsula, the species being light-demanding pioneers. All the species come very close together, and may have been derived from the breaking up of a single widespread ancestral type.

Compositae.

Though this great family is full of interest in its distributional aspects, as has been very fully demonstrated by the detailed work of Small (32), it must also be passed over with very brief reference. Schönland (30) finds several more or less well-defined groups of genera according to their distribution in South Africa, viz over 40 genera with general distribution, about 20 genera radiating from the Transvaal and Natal along the southern coast, over 40 genera mainly in the south-west, a small group chiefly central, and a few genera with very restricted distribution. There are also a very large number of species with extremely restricted distribution, as is to be expected in the family since it is a feature which it exhibits all over the world. On the other hand, many species are generally distributed, and many more have a wide range over one or more of the distinct climatic areas. Examples of our general principles may be found in the genera *Vernonia*, *Nidorella*, *Brachylaena*, *Sphenogyne*, *Pentzia*, *Cotula*, *Athanasia*, *Helichrysum*, *Metalasia*, *Stoebe*, *Othonna*, *Senecio*, *Euryops*, *Dimorphotheca*, *Osteospermum*, *Berkheya*, and many others.

Migration of widespread ancestral types has very clearly, in a great many cases, perhaps in the majority, been along the mountain ranges.

Ericaceae.

Schönland remarks (29) that the distribution of the Ericaceae defies at present a satisfactory explanation. 'Their prevalence in South-West Africa and the prevalence of Epacridaceae in Australia cannot be used as evidence that these two orders originated in the southern hemisphere from common ancestors (compare Drude in Engler-Prantl, iv. 1, 1897, 29).' We shall return to this point later.

There are 17 genera of Ericaceae with 146 species all endemic in the south-western region of Macchia. The genus *Ericinella* has one species at Graaff Reinet, one at Queenstown, and 3 or 4 in tropical Africa or Madagascar. The genus *Phillipia* has 4 species at the Cape, a few in tropical Africa, and 20 to 30 in the Mascarene Islands. The great genus

22 *Bewus.—Some General Principles of Plant Distribution as*

Erica has over 500 species, of which 469 are endemic in South Africa, mostly in the south-west, but about 20 in Natal, most of them, but not all, in the Drakensberg. *E. caffra* occurs even on the subtropical coast-belt, and several are common in the midlands. *Erica arborea* connects through the mountains of Central Africa with South Europe, where there are about a dozen species, none of which are South African. Other sections of the family are, of course, American and Himalayan, &c. These facts are mentioned because they would seem to have an important bearing on the origin of the South African flora, and they certainly do not count in favour of the supposed land connexions with Australia and South America. The enormous number of Cape endemics belonging to the genus *Erica* provides an interesting field for investigation. Why, for instance, are so many of them concentrated at certain points, e.g. the Cape Peninsula or the Van Staden mountains?

While we are dealing with facts of this general nature, I shall quote what Schönland (29) has said concerning one or two other families.

'The Penaceae and allied orders (the Thymelaeales) also fail to give us in their distribution an indication of their origin. Penaceae nearly allied to Thymelaeaceae; only distinguished by 4-merous ovary with 2-4 ovules each. All in South-West Cape Colony. 21 species and 5 genera.

'Geissolomaceae, 1 monotypic genus, closely allied to Penaceae, with which the order has frequently been united. South-west Cape Colony.

'Oliniaceae, fruit a drupe, while in the two previous orders the fruit is a capsule. About 6 species: 4 in Cape Colony, 1 in Usambara, 1 in Abyssinia.

'Thymelacaceae, with the exception of the polar regions, distributed over the whole globe, but many genera and groups very localized.

'*Peddiea*, 6 species. South-east and tropical Africa.

'*Gnidia*, 80-90 species. Tropical and South Africa, Madagascar, and East Indies.

'*Struthiola*, 24 species, mostly in South Africa, 3 in tropical Africa.

'*Cryptadenia*, 4-5 species. Cape.

'*Lachnaca*, 18 species. Cape.

'*Passerina*, 4 species, and *Chrymococca*, 1 species. Cape; 2 allied genera in central and northern Asia.

'*Dais*, 2 species; 1 in Natal, 1 in Madagascar.

'Elegnaceae. Chiefly in the northern, temperate, and subtropical zone: none in the southern hemisphere.

'The Bruniaceae, 12 genera and over 40 species, which are restricted to South Africa and even almost entirely to South-west Cape Colony, are so isolated among Saxifragaceae that we can also only point to their antiquity. About their origin no guess can be hazarded. . . .

'There are a number of genera of large orders which must be placed

in the same category, e.g. *Cliffortia* (about 40 species, mostly south-western Cape Colony), allied to *Bencomia* (a genus with 2 species in Madeira and the Canary Islands).'

The distribution of the Thymelacales and *Cliffortia*, &c., would surely favour a northern origin, and would therefore tell against Schönland's postulate of land connexions in the southern hemisphere.

We shall now deal with some of the families belonging to the Tubiflorae, since these illustrate present-day tendencies extremely well.

Acanthaceae.

Thunbergia atriplicifolia is a variable veld species widely distributed over the whole eastern side. Three other species (in addition to a whole series of varieties), viz. *T. aspera*, *T. xanthotricha*, *T. Bachmanni*, only differ slightly from *T. atriplicifolia*. *T. aspera* is distinctly more tropical, Natal coast-belt and northern Transvaal; *T. xanthotricha*, Transvaal, near Barberton only; *T. Bachmanni*, Transvaal, Orange Free State, and Pondoland. The discontinuous distribution of *T. aspera* between the coast-belt and the northern Transvaal is paralleled in many other cases in various families.

Good examples exactly similar to this may be found in the genera *Dyschoriste*, *Belpharis*, *Crabbea*, *Barleria*, *Justicia*. *Blepharis molluginifolia* extends from India through tropical Africa to Mossel Bay, being very common all over the eastern side of South Africa. *B. setosa*, which differs only in the scabrous leaves and setose bracteolés, is recorded only for the Natal coast-belt and tropical Transvaal. Similarly *B. boerhaaviaefolia*, which has the same distribution as *B. molluginifolia*, has given rise to several allied but apparently rare species in the tropics. *Crabbea nana* and *C. hirsuta* are very closely related and are both widely distributed grassveld species, an example of splitting or of A giving rise to B. *C. robusta* differs in being larger and having larger leaves and flowers. It is of a distinctly higher ecological type, and is more mesophytic; only recorded for Swaziland. *C. angustifolia* differs from *C. hirsuta* in minor characters. Distribution: Transvaal and Bechuanaland, endemic. *C. pedunculata* is nearly related to *C. nana*, and is endemic in Natal and rare. The whole family is very rich in examples like the above. The genus *Chaetacanthus* has four species, all endemic and all closely related. Two of them are widely distributed, two rare, an obvious example of the breaking up of an ancestral form. The Acanthaceae are a tropical family which have invaded South Africa, chiefly along the eastern side. They are peculiarly characteristic of the moist marginal belt in Natal coast-belt scrub and forest. The grassveld species and more xerophytic species generally are, however, more widespread in South Africa.

Since we have already sufficiently indicated how numerous the examples are in practically all families of the application of our general principles to species and varieties, we shall now make use of some members of the

Tubiflorae cohort to illustrate their application to higher groups. Guppy, as already mentioned, has indicated how they apply in the same way to the Geraniales, Geraniaceae, &c. The series now to be dealt with affords an even better example.

Scrophulariaceae.

First of all, the Scrophulariaceae are a distinctly cosmopolitan family, though, on the whole, most frequent in temperate regions. No fewer than twenty-one genera have a very wide distribution over South Africa. Most of the genera afford examples of widespread species giving rise to rare endemics, &c. And we also find examples of localized genera, 7 in the east, 3 in the south-west, 3 in the so-called Kalahari region, and 2 in the western. This in itself indicates how we may apply our principles of distribution to the genera as well as the species, but we can do still better if we follow the origin and distribution of a single well-defined tribe.

The tribe Selagineae of the Scrophulariaceae is sufficiently distinct to have been separated as a family by itself in the 'Flora Capensis', but it only differs in the characters of the ovary and fruit (the latter being two-celled or one-celled by abortion and indehiscent or separating into two one-seeded nutlets). Different authors are not quite agreed as to how much should be included in the tribe Selagineae, but if we take it in its widest sense then a polygenetic and polyphyletic origin from the Scrophulariaceae is extremely probable. It then consists of 10 genera and about 240 species, which are concentrated for the most part in South Africa. There are about 20 species in tropical Africa and one in Madagascar. There is an outlying genus (*Lagotis*) widely dispersed through the north temperate zone, another in the Mediterranean region (*Globularia*), and a single monotype in Socotra (*Cockburnia*) (3). The fact that *Lagotis* has been referred to the tribe Digitaleae by Wettstein (in Engler and Prantl) and *Globularia* retained in a distinct family (the Globulariaceae) shows that a polyphyletic origin for the tribe as formerly recognized has been made the grounds for breaking it up, but this attempt fails to give value to the marked peculiarities of the organs of fructification. It seems far better to recognize the Selagineae as a natural systematic group to which the Scrophulariaceae bears the same relationship as that between numerous widespread species and their derivatives as mentioned above. The widespread family A (the Scrophulariaceae) has given rise to a separate tribe (or another family) B, the Selagineae with a very restricted and at the same time discontinuous distribution.

We now select the two genera of the Selagineae, *Selago* and *Walafrida*. *Selago* has 112 species in South Africa, 2 of which extend into the tropics. There are also 17 endemic species in the latter region, mostly in the hills. In South Africa *Selago* is concentrated for the most part in the south-west (cf. *Heliophila*, *Oxalis*, *Pelargonium*, *Muraltia*, *Protea*, *Erica*, *Cliffortia*, and numerous other genera with a similar distribution). *Walafrida* is

quite closely related to *Selago* (the calyx has 3 lobes instead of 5 lobes). It has 31 species in South Africa, one of which extends into tropical Africa, 4 in tropical Africa, and 1 in Madagascar. It has a similar distribution in South Africa to *Selago*, but on the whole rather more eastern. The two genera, being so nearly related, obviously may be regarded as the result of divergence from a common ancestral type, like so many nearly related species already dealt with. The genus *Hebenstreitia* is more distinct. Of the thirty species, most of the annuals are central or western, i.e. in the drier parts, while the perennials are widespread or eastern. One species extends as far as Abyssinia. All the species of *Dischisma* (11), *Microcodon* (5), *Agathelpis* (3), and *Gosela* (1) are endemic in the west or south-west.

Lastly, as regards the species, a few examples will suffice. *Hebenstreitia fruticosa*, *H. dentata*, and *H. integrifolia*, all three of which are very variable and come close together, occur all over South Africa. *H. Watsoni*, on the other hand, is only recorded for East London, and only differs from *H. integrifolia* in its more lanceolate and acuminate bracts. *H. Rehmanni* (Transvaal high-veld only), again, differs in its small flowers and very spreading bracts. *Selago* supplies numerous examples of the same kind.

The process of differentiation from the Scrophulariaceae, which we have just followed through the Selagineae (or Selaginaceae), might also be followed through the Myoporaceae, which Baillon ('Hist. Pl.,' ix. 420) again reduces to a tribe of the Scrophulariaceae. The genus *Oftia* has two species, both endemic in the western and south-western regions of South Africa. There are 6 genera in the family and about 80 species, mostly Australian, with a few Polynesian representatives; 1 in the Sandwich Islands, 1 in Mauritius, 2 in China and Japan, 1 in the West Indies, 1 somewhat doubtful in tropical Africa, and 2 in South Africa.

Interesting points might also be brought to light by a comparison of the Scrophulariaceae and Bignoniaceae; in fact, the whole of the Tubiflorae affords an excellent field for the study of differentiation and distribution.

Verbenaceae.

This family is chiefly tropical in both hemispheres. In South Africa the primitive tribe Stilbeae, which has endospermic seeds and in three of the five genera regular corollas, is entirely endemic in the south-west. In the tribe Verbeneae, on the other hand, which has exendospermic seeds and zygomorphic corollas, one genus, *Bouchea*, which extends through tropical Africa, America, and India, is widespread over South Africa, and the others are all eastern. The tribe Viticeae, which differs in having the ovule inserted laterally instead of being basal erect, has two genera, *Vitex* and *Clerodendron*, each with nine or ten species, mostly rare in Natal and Transvaal endemics except *Clerodendron glabrum*, which extends from Grahamstown

26 Bews.—Some General Principles of Plant Distribution as

into the tropics, and *C. myricoides* and *C. spinescens*, which are also tropical.

Laurineae.

The genus *Cryptocarya* has over forty species in the tropics (mostly Indo-Malayan). There are six species in South Africa. *C. Woodii* is widespread over the eastern side. The species *C. latifolia*, *C. myrtifolia*, *C. Sutherlandi*, and *C. Wyliei* are all allied to *C. Woodii*, and are all rare Natal or Zululand endemics. *C. Woodii* is replaced in the south-west by a more xerophytic, more distinct, shrubby species with narrow leaves, *C. angustifolia*.

Proteaceae.

This family, as is well known,* is concentrated mostly in the south-west, but, as a family, its distribution is similar to many genera already dealt with; it has certain eastern extensions. *Protea hirta* is fairly common all over Natal, extending down even to Durban on the subtropical coast-belt. There are about nine or ten other species, mostly on the Drakensberg, where they are dominant in the extensively developed Protea-veld (4). The genus extends along the mountains of tropical Africa to north of the Equator. The genus *Faurca* is typically eastern and tropical, with one species in Madagascar. Incidentally it again illustrates our general principles in that *Faurca saligna* is very widespread and sometimes dominant in tree-veld, while several other species, e.g. *F. natalensis*, *F. Macnaughtoni*, are very rare. The last-mentioned also shows discontinuous distribution. The northern and eastern extensions of the Proteaceae are emphasized because this family is typical of those which are used to demonstrate the connexions between South-West Africa and Australia. The 960 species are distributed as follows: 591 in Australia, 25 in tropical East Africa, a few in Angola, 2 in New Zealand, 7 in Chili, 36 in tropical South America, 262 in South-West Cape Colony, 2 in Madagascar, about 5 on the mountains of tropical Africa.

The Monocotyledons.

Here we find the same principles illustrated as among the Dicotyledonous families. The bulbous Monocotyledons may be looked upon as a type peculiarly suited to the variable veld conditions in South Africa in all the climatic areas, where they have multiplied enormously, but though there are a great many endemic South African genera, especially among the Iridaceae, they are by no means poorly represented in other parts of the world. The great southern genus *Moraea*, which is spread over South Africa, tropical Africa, Madagascar, and Australia, is hardly different from the northern genus *Iris*. *Romulea* extends to the Mediterranean, *Gladiolus* to Europe, &c. There are connexions with South America and Australia on which considerable stress has been laid by various writers. For details see Schönland (30). But the connexions with the north appear equally

notable. The divergence is always considerable in the case of the Australian and South African and South American connexions. The species and even the genera are usually distinct. In general the distribution of the bulbous Monocotyledons can be explained equally well by divergent invasion from the northern hemisphere as by land connexions in the southern.

As regards the South African species and their present-day distribution, practically all the large genera show relatively wide-ranging species around which relatively rare endemics may be grouped; or allied species in neighbouring areas or under different ecological conditions; or variable species in the process of breaking up. Being of a rather uniform ecological type, however, the examples are not quite so striking as when a xerophytic, widespread, shrubby species among the Dicotyledons is connected with a mesophytic forest species. • The bulbous Monocotyledons nearly all belong to early stages of the plant succession.

• Among the Liliaceae, the genus *Asparagus* is more varied in its ecological behaviour, and widespread species may be paired with rare endemics in a very striking way, e.g. *A. declinatus* and *A. Macowani*; *A. stipulaceus* and *A. Burchellii*; *A. striatus* and *A. erectus*; *A. sarmentosus* and *A. oxyacanthus*; *A. falcatus* and *A. Sprengeri*; *A. aethiopicus* and *A. myrioclados*; *A. medioloides* and *A. Kraussii* and *A. colubilis*. The large genus *Aloe* similarly supplies numerous examples of the working of our general principles.

Orchidaceae.

There are eighteen endemic genera, of which one, *Huttonaca*, is eastern and the rest mainly or entirely south-western. Another twenty-eight genera are also found in tropical Africa, and these have invaded South Africa to a greater or less extent from north and east towards the south-west. For details concerning the distribution of the genera and other Monocotyledonous families, Schönland should be consulted (30).

Restionaceae.

As is well known, this is another of the typically south-western families, only two species (one each of *Restio* and *Leptocarpus*) being found in Natal. The family may, however, probably be connected with the Eriocaulaceae, which are much more widely distributed in the tropics of both hemispheres.

Gramineae.

The 500 odd species of grasses in South Africa fall naturally into two distributional groups, the south-western being distinctly separated from the rest. The south-western grasses are temperate in their affinities, the eastern, northern, and western are tropical. Eighty or ninety of the south-western species are endemic. The chief genera are *Danthonia*, *Pentaschistis*, *Pentameris*, *Achneria*, *Avenastrum*, *Brizopyrum*, *Lasiachloa*, *Ehrharia*. Though the temperate tribes are thus mostly concentrated in the south-

west, yet along the central and eastern mountain-ranges we have many representatives (there are twenty or so on the Drakensberg), and some even compete with subtropical species in the eastern grass-veld, e.g. *Danthonia purpurea* around Molteno and *Pentaschistis natalensis* in Natal. As Stapf (33) has pointed out, temperate species of *Avenastrum*, *Agrostis*, *Melica*, *Poa*, *Festuca*, *Bromus*, *Brachypodium*, form, as it were, a bridge across the mountains of the tropics connecting with the northern centres of distribution. The south-western section of the Gramineae, then, like most of the other characteristic south-western families and large genera, are not without representatives eastward and northward.

The Gramineae of the great eastern grass-veld region and of the central and western regions have much more obviously invaded South Africa from the tropics. The genus *Aristida* has produced species, many of them rather closely connected, e.g. *A. congesta* and *A. barbicollis*, the former very common and widespread, the latter much rarer, which are of supreme importance in the early stages of the establishing of grassland. Other similar pairs are *A. angustata* and *A. junciformis*, *A. bipartita* and *A. Burkei*.

Aristida has also a very distinct section (*Stipagrostis*), the species of which are adapted to desert and semi-desert conditions of the dry central and western areas. No better example of differentiation could be found anywhere than that of *Aristida* in South Africa.

Eragrostis is another great pioneer genus in grass-veld, with about fifty species. Many of the rare Transvaal and Free State endemics (*Eragrostis barbinodis*, *E. pallens*, *E. Wilmsii*, *E. sporoboloides*, *E. Atherstoni*, *E. echinochloidea*, *E. margaritacea*, *E. dura*) may very well have been derived from the widely distributed species, *E. curvula*, which is so widespread and abundant everywhere. Except for the shorter valves, the differences are in no case very great. Numerous similar examples could be given from the Andropogoneae, which are characteristic rather of climax grassland. *Andropogon hirtus*, for instance, has probably produced *A. auctus*; *A. nardus* is in the process of breaking up into several distinct species, and has already given rise to *A. plurinodis*. The variations of the dominant grass, *Anthistiria imberbis* (*Themeda Forskalii*), should be studied from the same standpoint. The numerous mesophytic species of *Panicum* and other Paniceae which are characteristic chiefly of the moist marginal belt surrounding forest and scrub are of interest, since some of them show discontinuous distribution, particularly between the Natal coast-belt and the northern Transvaal. Many of them, however, extend into the tropics and have simply invaded South Africa at different points. I regret that space does not permit of a fuller discussion of the Gramineae from the standpoint of the present paper. In fieldwork, I have recently found that the presence of many species in certain grassland areas can be explained only on these lines, i.e. by taking into account their probable origin from other widespread

species. Otherwise none of the ordinary ecological methods could account for their presence in one place, their absence in another.

Coniferae.

The genus *Podocarpus* is of great interest, not only because it has been found in the fossil Tertiary deposits of northern Europe and America, though it is now confined to Japan and the Southern Hemisphere and reaches such widely separated points as New Zealand, South Africa, and southern Chili, but also because species are so often dominant in the chief South African forests.

The two species *Podocarpus latifolia* (*P. Thunbergii*) and *P. elongata* are quite distinct and have, more or less, the same range. They are both old species; *Podocarpus falcata* is closely allied to *P. latifolia*, and it is probably identical with, or at any rate extremely close to, *P. melanianthus*. *P. falcata* is completely dominant in forests of a narrow belt along the southern end of the Drakensberg, but is apparently as completely absent from exactly similar habitats north of the Mont aux Sources. If it is the same species as *P. melanianthus* it begins again in Rhodesia and East Africa, and we have a striking example of discontinuous distribution in a dominant forest species. If it is not the same species as *P. melanianthus*, we have a case of two derivatives from the same species differing slightly in two distinct areas.

ISOLATED TYPES.

The above survey of families, genera, and species has now been made of sufficient length to show that the flora of South Africa affords abundant evidence of the origin of new species, according to the principles summarized to begin with. If I had cared to utilize all the notes that have been prepared the survey could have been extended to at least three times the length. While in the larger genera it is often very easy to see the nearest relationships and probable origin of rare endemics or the splitting up of variable species, &c., there are, on the other hand, many isolated species or genera, or even families (e.g. *Burseraceae*), to which at the present stage of our knowledge it is impossible to apply those methods. Many examples are to be found among our forest-trees and shrubs, e.g. *Ocotea bullata*, *Pygeum africanum*, *Canonia capensis*, *Clausena inaequalis*, *Colodendron capense*, *Ilex mitis*, *Noltea africana*, *Hippobromus alata*, *Curtisia fragina*, *Heteromorpha arborescens*, *Leucosidea sericea*, *Canonia capensis*, *Platylophus trifolius*, *Choristylis rhamnoides*, *Xymalos monospora*, *Gerrardiana foliosa*, &c., which have no obvious connexions in South Africa, though several of them do have such in the tropics. In the latter case, their distribution in South Africa must be explained by the ordinary methods of migration. They must be assumed to have migrated into South Africa by themselves. They are not the descendants of immigrants. In the case

of endemic monotypes with no obvious close relationships the difficulty of reaching a satisfactory explanation is greater.

Fortunately there are not many such, and several which were once thought to be of this type have since had relations discovered in the tropics. If all other explanations fail, they must be considered relicts, whose immediate ancestors have disappeared. Guppy would class most of them as specialized types, the oddities of plant forms which have been produced not by differentiation from a more generalized type but by increased specialization. The Rosaceous *Pygeum africanum* or *Leucosidea sericea* are as good examples as any, but *Noltea africana*, *Hippobromus alata*, *Curtisia faginea*, are also monotypes.

Other isolated types have been referred to in the general survey of the families. It may be noted incidentally that monotypes and genera containing few species have generally more restricted distribution than others. (Cf. de Candolle, 'Origin of Cultivated Plants', 2nd edit., 1909, p. 395, in connexion with the origin of *Zea Mays*.)

GENERAL APPLICATIONS.

My object in bringing forward these facts and arguments has been mainly to afford illustrations of what I have begun more and more to realize in connexion with field ecological work, namely, the necessity for new view-points. The ordinary ecological methods of investigation are extremely useful up to a certain point. We find that certain plants occupy certain habitats, each with a certain range of the measurable factors, which vary to a considerable extent independently. As Clements has pointed out, and as can be seen by any one who has studied plant-life in the field, very fine measurement of each factor may be largely wasted effort, since (with the possible exception of some soil factors) it requires a relatively large difference in any single factor to cause any morphological adjustment. Nevertheless, since the factors vary independently, there are a fair number of permutations permissible. Thus as regards the moisture factor, habitats may be divided into aquatic, semi-aquatic, moist, mesophytic, dry and very dry, and any of these may be combined with habitats having full sunlight, diffuse light, and dense shade, and then with clay soils, sandy soils, and so on. In spite of their seeming complexity the essential inorganic differences between habitats are not so very difficult to recognize. Biological factors, however, are more complex, and ecologists are giving more and more attention to them. In its widest sense, this aspect of ecology includes the vegetation itself as a factor and embraces, therefore, the whole question of plant succession.

We are by no means near the end to be reached by the use of these ecological methods, and how very useful they are has already been shown by the results, but if we do not take into account the historical or geo-

graphical or evolutionary factor, whatever we care to call it, we are neglecting another very useful method of attack, and we fail to explain phenomena as fully as we might. The dominance of *Podocarpus falcata* in certain Drakensberg forests cannot be explained by considering habitats alone, for it is absent from apparently identical habitats in other parts of the range. The reason becomes clear when we consider its origin from the widespread *P. latifolia*. The presence of a mesophytic species of *Rhus* in a patch of moist scrub is not due simply to the fact of the more mesophytic conditions alone. We must connect its presence there with that of a widespread parent species over drier areas. In a word, it is not the present species alone that we must study, but wherever possible we must go back a step or two and consider allied or parent species and their behaviour and distribution.

The whole of our argument is based on the hypothesis that a species in the course of its migrations, when it comes into contact with conditions different from those which produced it, is, in many cases at least, capable of differentiation or of giving rise to new species suited to the new conditions. In a larger sense, and over a longer period of time, the same applies to larger groups, the genera, tribes, and families. While, in this paper, effort has been directed chiefly to bringing forward evidence of as varied a nature as possible to show that this takes place, there are many other interesting points which have not been dealt with. What conditions, for instance, are most effective in producing new species? Is the passage from tropical to temperate conditions more important than from dry to moist (or vice versa in each case)?

The examples of tropical species being replaced in South Africa by nearly related more temperate species are very numerous, but this is to be expected from the size of the areas involved. On the other hand, the examples of xerophytic shrubby species being closely allied to mesophytic forest species are also very numerous. The transition from siliceous to calcareous soil conditions has been closely investigated in Europe from this standpoint, but few observations have been made in South Africa on the effect of differences in soil conditions.

While the ecological aspects of this study are what have been kept most in view the methods of studying present-day tendencies obviously have a bearing on the question of the origin of the South African flora as a whole and its connexions with outside areas. From what was said in the introduction, it is clear that there are only two general views to be considered.

One lays emphasis on the South African connexions with the floras of Australia (particularly South-West Australia) and of South America and postulates land connexions to explain them, across what are now deep ocean basins. The difficulty with hypothetical changes of sea and land on a vast scale is always, as Wallace has expressed it again in his last work

(1911, 36), that they create more serious difficulties than they are supposed to explain. After a half-century of thought and work on this and allied subjects, Wallace had lost none of his vigour in denouncing theories of continental extensions. He concludes by saying, 'I believe it can now be truly said that no stratigraphical geologist accepts the theory of frequent interchanges of continental and oceanic areas, which are so hastily claimed by palæontologists and biologists to be necessary in order to overcome each apparent difficulty in the distribution of living or extinct organisms, and this notwithstanding the number of such difficulties which later discoveries have shown to be non-existent'.

The other view of the origin of the southern floras is that of Thiselton-Dyer and Guppy, which has already been fully explained. A northern origin for many important elements of the South African flora has of course always been admitted. With regard to the south-western flora, a sufficient number of families and tribes, e.g. Proteaceae, Ericaceae, Rutaceae, Verbenaceae, Restionaceae, Gramineae, Selagineae, and large genera, e.g. *Heliophila*, *Muraltia*, *Pelargonium*, *Oxalis*, *Erica*, *Cliffortia*, *Selago*, *Danthonia*, *Pentastichis*, and many others, have been dealt with in our general survey to show that in practically every case, while the greatest concentration is in the south-west, and within that region in such 'cul-de-sacs' as the Cape Peninsula, yet there are eastern and northern outliers or relatives which in many cases extend right through the tropics to Europe and Asia.

It must of course be admitted that these are very meagre when compared with the rich development of species in the south-west, but at the same time, there are no real grounds for the common assumption that the area where a family or genus is now best represented is necessarily the centre where it has originated. If the primitive ancestors of the south-western flora came from the north, there is no need to assume that the original immigrants were very numerous. They have left few descendants along the track of their invasion because conditions there are not generally suited to them, but when they reached more temperate areas in South Africa they multiplied exceedingly and produced many divergent types in the course of their differentiation.

One reason, perhaps the chief reason, why the second view appears the more acceptable is that it represents conclusions which can be reached by applying the principles which we can study at the present day within South Africa with regard to the origin of species. If we simply extend the time and the area, we can apply the same principles to the genera, tribes, and families, and the example of the origin of the Selagineae, at present mostly South African, and in South Africa mostly south-western, from the widespread Scrophulariaceae clearly throws light upon what has happened during the past.

The view-point adopted in this paper is also a useful one in connexion

with the general study of the phylogeny and inter-relationships of the Angiosperms, as has been demonstrated by Small in connexion with the Compositae (32). Morphologists and systematists are far from agreed on which characters in the flower are to be considered really primitive. If widespread species or genera or families have given rise to types with more restricted distribution and this is found to hold generally, the deductions are obvious. The hypothesis of course requires much testing, but it may serve to throw light on the question of how evolution has progressed and whether it has been mainly from the simple to the complex or vice versa. A comparison of some of the examples quoted in the general survey above, from this standpoint, would yield interesting results. Take, for instance, the genus *Eragrostis* among the grasses: *Eragrostis curvula* differs from the numerous endemic species which can be grouped round it chiefly in having longer valves. A shortening of the valves would appear, therefore, to be one of the evolutionary tendencies in this genus. Again, if we take the Gramineae as a whole, it is remarkable that the south-western genera—the older types—belong mostly to tribes (e.g. *Aveneae*, *Festuceae*) which have numerous florets in the spikelet, often much exerted from the glumes, while the presumably more recent invasion of grasses into South Africa, as represented by the eastern grass-veld genera, consists of types which, as a rule, have only one perfect floret in the spikelet (e.g. *Andropogoneae*, *Panicaceae*). Similar facts have been briefly touched upon in respect to other families, but further work is necessary from this standpoint, as from the others. From all standpoints, the South African flora affords excellent materials and opportunities for the study of plant distribution.

SUMMARY.

1. Of those who have dealt with plant distribution, Darwin and Wallace objected to invoking geographical change as a solution of every difficulty, while Hooker was more inclined to postulate continental extensions to explain the connexions between the floras of the southern hemisphere. Schönland, after carefully considering the facts, finds a land connexion between South Africa and Australia and between West Africa and America to afford the simplest explanation. Threlton-Dyer and Guppy agree on a theory of southward migration from the northern hemisphere. Guppy also elaborates a theory of differentiation, which he applies to families, tribes, genera, and sections of genera. These each tend to fall into two groups, primitive and derivative, the first widespread, the second restricted in area. Willis has developed the 'age and area' theory based on the fact that endemic species in any area tend to have a narrow range, non-endemic species a wider. The older a species is, the wider its range. Engler, Drude, and Clements agree that 'multiple origins' are possible, the former admitting the possibility chiefly in the case of larger groups, but Clements applying it also to species. The writer in

34 *Bews.—Some General Principles of Plant Distribution as*

this paper considers present-day tendencies in plant distribution in South Africa, directing attention chiefly to the origin and distribution of species, but extending the observations also to larger groups.

2. The present-day conditions in South Africa are summarized and the climatic areas are arranged in increasing order of mesophytism: (a) Western region, (b) Central Karroo region, (c) Cape or South-western region, (d) Sandveld region of the Kalahari, (e) Thornveld areas of the eastern side, (f) Highveld and mountain areas of the eastern side, (g) Coast-belt of the eastern side.

3. Traversing all these areas are certain primitive habitats which are occupied by widespread species: (a) Cultivated land, waste places, &c.; (b) lakes, streams, marshes; (c) sea-shore habitats, mostly sandy; (d) mountain ranges; (e) drier areas of various kinds. • To the north a fairly uniform tropical zone crosses the continent and allows tropical species to invade South Africa at different points.

4. A widespread species in the course of its migrations, when it comes into contact with conditions different from those which produced it, is often capable of giving rise to a new species suited to the new conditions. In South Africa, at the present time, such derived species are usually more mesophytic than the parent species, but they may be more xerophytic. In many cases, tropical species give rise to temperate species. One widespread species may give rise to several derived species or may itself break up into several, and, in many cases, the same derived species may have widely discontinuous distribution while its parent form is common all over the same area, a fact which suggests the possibility of 'multiple origins' or polygenesis.

5. Certain objections to the evidence for this are discussed and then a large number of examples are recorded. These are drawn from as many different families as possible, and but for consideration of space could have been largely increased. The cohort Tubiflorae is used to demonstrate that the same principles may be applied to larger groups, the widespread family the Scrophulariaceae having produced the Selagineae chiefly confined to South Africa but with outliers elsewhere, and similarly the Myoporaceae in Australia, &c. Also attention is directed to the distribution of characteristic south-western genera and families and their eastern outliers or connexions as throwing light upon the question of the origin of the South African flora.

6. In conclusion, general applications are discussed with special reference to (1) plant ecology, (2) the origin of the angiospermous flora of South Africa, (3) general phylogeny of the Angiosperms.

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Anatomy of the Ovule and Seed in *Gnetum Gnemon*, with Notes on *Gnetum funiculare*.

BY

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With Plate I and five Figures in the Text.

G. Gnemon.

SOME time ago I had the good fortune to examine inflorescences of *Gnetum Gnemon* bearing a few large seeds and a number of small abortive ovules, which Professor W. H. Lang kindly placed at my disposal, and later Dr. E. N. Thomas was so good as to hand over to me two seeds of an intermediate age, a little larger than those described by Miss Berridge.

To recapitulate the structure of the female flower:—As in all species of *Gnetum*,¹ there are three envelopes, the outer of which is succulent when ripe and is regarded here as probably foliar in nature; the middle is complex in structure, and at its tip has an angled fibrous zone, and is here regarded as the outer integument, whilst the inner covering (or inner integument) is prolonged into a freely projecting micropylar tube. Miss Berridge in *Gnetum Gnemon*, and the author in *Gnetum africanum*, have drawn attention to the closure of the lumen in the micropylar tube and the outgrowth of tissue from its wall forming a flange projecting over the tip of the middle covering.

The new material has enabled me to follow in detail the earlier stages in the closure of the lumen of the micropylar tube and the outgrowth of tissue from its wall, while providing a most useful opportunity to investigate further remarkable changes which occur in the older seed, resulting in such a complicated and altered mature structure as could not have been anticipated from the study of the earlier stages. The mature structure for the first time described presents points of interest which emphasize the comparison already made with the Bennettitalean seeds.

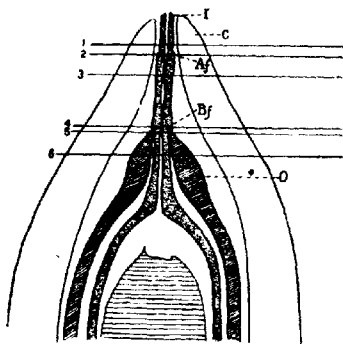
Early stages. Of the numerous small ovules which I examined, nearly all show some signs of the outgrowth of the cells lining the middle and lower portion of the micropylar tube.

¹ Strasburger, 1872, 1879; Lotsy, 1899; Berridge, 1911; Thoday, 1911; Pearson, 1915.

Text-fig. 1 is the diagrammatic representation of the top part of a young ovule in which the changes have begun. The figure is built up from longitudinal sections connected with the series of transverse sections figured in Photos 1-6, Pl. I.

From the opening in the thick fleshy outer covering (c) freely projects the open tip of the micropylar tube. The micropylar canal is still open throughout, though at two places it shows signs of narrowing. The wall of the micropylar tube is already growing in thickness, and at *Af* and *Bf* are seen projecting portions. Into *Bf* fits the tip of the middle covering. The details of this figure are better explained by reference to the photographs

and descriptions of the transverse sections.



TEXT-FIG. 1. Diagrammatic view of section of young ovule of *G. Gnemon*, 3 mm. long, in which closure of micropyle is just beginning. *c*, outer covering; *o*, middle covering; *i* is the inner covering terminating in the freely projecting micropylar tube. *Af*, *Bf*, are the beginnings of the projecting upward and downward flanges of the micropylar tube. 1-6 represent the levels of Photos 1-6, Pl. I.

micropylar tube, a thin ring of tissue one cell thick (*f*, Photo 2, Pl. I). When this is traced downwards it is found to be an upward continuation of the papillate outer epidermis of the micropylar tube (*p*, Photo 2, Pl. I). That is, it is formed by upwardly directed papillae, closely appressed laterally (Text-fig. 1, *Af*). It is the beginning of the upper 'flange' described in an older ovule by Miss Berridge; in her drawing of a longitudinal section¹ she figures rows of cells directed *upwards*, which must have had their origin as now described. In the region below these freely projecting hairs, the wall of the micropylar tube is about 6-7 cells thick, and though the outer epidermis is fringed with prominent papillate cells they have not projected far out into the cavity between the micropylar tube and the outer covering (Photo 3, Pl. I).

At the level of the upper flange, the lumen of the micropylar tube

¹ Berridge, 1911, p. 112.

becomes triangular (Photo 2, Pl. I), because of the ingrowth of the cells of the columnar epidermis; here one or two of these cells, and a little lower many of them, are transversely divided (Photo 3, Pl. I). Farther down, the lumen widens for a short space, and becomes a narrow oval, but very soon it narrows again (as seen in Text-fig. 1). This transitory wider lumen only shows that the changes which accompany closure begin first or proceed fastest at the upper and lower ends of the thickened region of the micropylar tube. In the lower part of the thickened region the lumen is quite narrow, is filled with mucilage, and its epidermal cells are both greatly elongated and subdivided into rows; the sub-epidermal cells also are divided radially. The papillae produced by the outer epidermal cells project more freely outwards, and some of the cells below this outer epidermis are dividing radially.

The next section figured (Photo 4, Pl. I) cuts the tip of the outer integument (middle covering), which is buried in the hairs at the base of the thickened region of the micropylar tube. The tip of the outer integument is fringed with papillate cells with thickened, often slightly lignified, walls, which tend to aggregate into groups, and are shown in the photo at X. The hairs of the edge of the inner integument are often prolonged between them so that they appear in section as free groups of cells surrounded on all sides by hairs. It is difficult or impossible even at this stage to make out clearly the line of separation between the tip of the outer integument, micropylar tube and hairs; the epidermis of both coverings being papillate, they fit closely together.

Some way below the level of the tip of the outer integument the lumen of the micropylar tube widens, and the elongated lining cells gradually resume their regular shape. The lumen is at first triangular (Photo 6, Pl. I) and then enlarges to form the large cavity surrounding the apex of the nucellus. Into this cavity projects the shrivelled tip of the nucellus with its indurated walls (Photo 16, Pl. I), to me reminiscent of Prof. Seward's description of the small nucellar beak in *Williamsonia scotica*.¹

In this ovule there is no sign of lignification of any of the tissues of the outer integument, the inner layer (which ultimately develops into a strongly lignified zone of cells like that described in *G. africanum*) being here composed of small closely compacted cells, isodiametric in transverse section. There are already signs of radial growth on the shoulder of the outer integument to form the expanded region found in the older seed. The other small ovules examined by transverse section differed only in detail from the one chosen for the above description.

Other small ovules were cut in longitudinal section. One showed as yet no sign of the formation of a flange or thickened region or of the

¹ Seward, 1912, p. 112.

ingrowth of the inner epidermis of the micropylar tube. Photo 15, Pl. I, shows the tip of this ovule.

Each of the others had a small flange composed of a few hairs in which the tip of the outer integument was buried. In one of these the lumen of the micropylar canal was still open, although the ingrowing epidermal cells had almost everywhere divided once and the epidermal layer three or four times. In another the flange was more developed and the wall of the tube thicker. The epidermal cells that grow out to form the flange had already divided into radial rows, as figured by Miss Berridge.¹ The middle covering in this ovule had thickened and the formation of the expanded shoulders was already beginning.

In these young ovules there is much mucilage, both in the lumen and in the cavity between the micropylar tube and the nucellar apex. The nucellar apex is composed of rows of cells radiating outwards, and degenerating in the region of the pollen-chamber. Photo 16, Pl. I, shows the degenerating tissue above the radial rows of cells in the nucellar apex.

Seeds about 8 mm. long. The two seeds about 8 mm. long were only a little more advanced than the stage described by Miss Berridge (1911), and their structure is easily comparable with that shown in her Fig. 1. Near the apex of the micropylar tube the upwardly directed tissue growing out from the wall of the micropylar tube was more sharply marked than in Miss Berridge's Fig. 1, and appears as a free ring in transverse sections above the level of its origin from the tube. In this region the proliferated wall of the now closed micropylar tube is composed of clearly distinguishable and beautifully regular rows of cells, and is brought closely into contact with the outer covering; indeed in some places their boundaries are indistinguishable. The growth in thickness of the micropylar wall as a whole has resulted in the closing both of the cavity between it and the outer covering and the lumen itself, which is now quite obliterated.

In the lower part of the closed portion of the tube the wall is narrower and the cavity tends to reappear, but is blocked by a solid mass of proliferated epidermal cells which have grown out more irregularly as papillae with enlarged ends and are now strongly lignified, thus forming a very conspicuous rod of cells, something like, though much more developed than, the core described in *G. scandens*, and figured in a former paper.²

Just below this papillate region the open tube has a cuticularized lining; near the bottom of the lower flange the lining is torn, but the tube is nowhere broken across.

When the level is reached at which the tube is supported by the tip of the middle covering the lining is again entire and cuticularized. The tip of the middle covering is still buried in the hairs of the downwardly projecting flange.

¹ Berridge, 1911, p. 149.

² Thoday, 1911; Fig. 9, Pl. LXXXVI.

The ovules show the structure of the middle part of the seed more satisfactorily than do the older seeds, where more lignification has made sections of this part almost unattainable. The extreme tip of the outer integument is parenchymatous, but a little lower a band of lignified tissue appears on its inner edge (Photo 14, Pl. I), and as soon as the terminations of the vascular bundles are reached this band becomes rayed, each ray projecting opposite a vascular bundle. At first there are four or five vascular bundles, and four or five rays to the fibrous band as described in *G. africanum*, in a former paper,¹ but a little lower a large number appear in the sections. Near the apex of the middle covering the cells of the thick-walled tissue, and also of the outer parenchymatous tissue, which is here expanded to form the widened shoulders of the seed, are radially elongated and arranged in radial rows, and a palisade layer is visible between the thick- and thin-walled cells, but this soon becomes irregular. Below the shoulders the thickening of the walls almost ceases at this stage, and they are no longer lignified, though a differentiation into inner and outer layers is visible throughout.

The oval pointed fleshy seeds of *G. Gnemon* have been figured by Lotsy,² but the seeds here described in detail were of much greater size.

The mature seed. Text-fig. 2 A is drawn to scale from a mature seed of *G. Gnemon* 3.3 cm. long. Half of the outer covering has been pared away, leaving exposed the outer integument, which is distinctly angled.

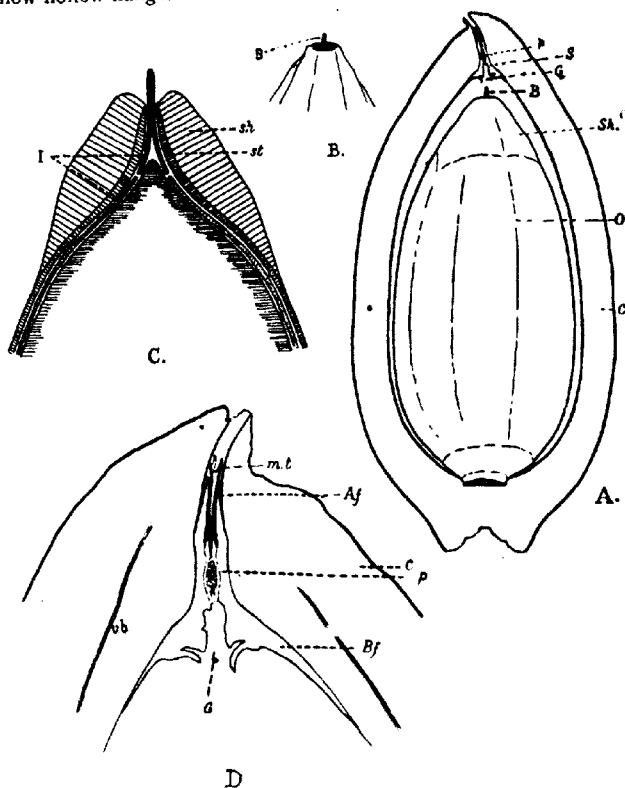
The apex of the closed micropylar tube is here seen to be fused with the outermost covering, forming a sort of stopper. At the same time the tip of the middle covering (O) is no longer fused with the lower flange of the micropylar tube, but the outer covering has grown up and carried with it the 'stopper', breaking the micropylar tube across at its weakest point. The apex of the middle covering is now at a considerable distance below the stopper and a bit of broken micropylar tube (B) sticks out from it.

A section of the apex of the seed is seen more highly magnified in Text-fig. 2 D. The indurated open tip of the micropylar tube, which is unusually long in this particular specimen, yet does not extend to the surface of the outer covering, is seen in section surrounded by a long upwardly directed flange (Af) which has grown out from the wall of the tube lower down. The outer surface of the wall below this flange is fused with the outer covering. In this region the wall of the tube is thickest, and the lumen is filled with thin-walled closing tissue (p), derived from the ingrowth of the epidermal cells. Below this thin-walled tissue there is a gap (G) with a torn irregular lining; here part of the wall of the tube alone is left fused to the outer covering, the inner part of the tube with its mass of closing tissue having been dragged out when the tube broke across (micropylar beak, Text-fig. 2 A), owing to the greater growth in length of the outer

¹ Ibid., p. 1115.

² Lotsy, 1899.

covering. The section shows the great width of the lower expanded part of the now hollow flange.



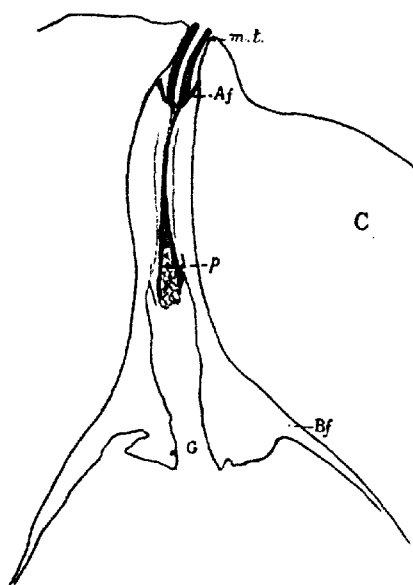
TEXT-FIG. 2. Drawn from mature seed of *G. Gortoni*, 3 cm. long, described in text. A is the whole seed enclosed in inner and middle coverings seen *in situ*, with half the outermost covering pared away to show the top part of the micropylar tube, the stopper (s), in which p is the closing tissue, and g the gap out of which the beak (B), the bottom part of the micropylar tube, has been torn. C is the outermost covering, O the angled outer integument composed of an inner hard layer and an outer soft layer, the latter expanded at SA over the shoulders of the seed ($\times 4$). B is the apex of the outer integument looked at from above, showing the beak (B) projecting from a depression ($\times 5$). C is drawn from the median section of B. The nucellus, filled with endosperm, is seen enclosed firstly by the inner integument (I), parenchymatous below (dotted area) and lignified above (blackened beak); secondly, by the tip of the outer integument, with its expanded soft outer layer (sh) and stony inner layer (st) ($\times 10$). D is the apex of A more highly magnified ($\times 18$), and shows the tip of the outermost covering (C) with the stopper. Af and Bf correspond to the upward and downward growing flanges of the micropylar tube in Text-fig. 1. mt = the tip of the micropylar tube; p = closing tissue; g = gap; v.b. = vascular bundle.

Text-figs. 2 B and C are taken from the apex of the middle covering (outer integument) of the same ovule, and show the broken portion of the micropylar tube (B) projecting upwards, both as seen in the solid object and

in section. Minute examination of the top part of this beak-like structure shows its irregular outer surface corresponding to the irregular inner surface of the cavity (G) from which it was torn; it is heavily lignified throughout, and the lignification extends (Text-fig. 2 C) down to the level opposite the nucellar tip, below which the inner integument is parenchymatous. The tip of the middle covering is also seen in this figure with its stony inner layer (*st*) and soft expanded outer layer (*sh*).

Text-fig. 3 represents a longitudinal section of another seed in which the tip of the micropylar tube reached the surface of the outer covering. Below the open portion of the tube with its thickened cuticularized lining and upwardly directed short corky flange, there is the widened part fused to the outer covering. In this seed a strand composed of a few of the thickened cuticularized cells forming the original lining of the micropylar tube could be traced down the middle of the solid portion; lower down, this widened as the part of the tube at an early stage more widely open, but now filled with papillae, is approached. A few thylose-like papillae are still left, unlike the other seed in which they had all been torn out by the breaking away of the rest of the tube.

Cross-sections show even more clearly that what has been torn away is the solid closing rod of papillae formed from the epidermal lining of the tube, and with it some of the sub-epidermal layers, the part of the wall formed by subdivision of the outer layers being left. In a series of transverse sections, taken from above downward, through another mature seed very like the last, the first few sections cut through the outer covering with its round excentric lumen, limited by a definite epidermis, and with the free micropylar tube projecting through it (Photo 7, Pl. I). The latter is composed entirely of thickened cells with a strongly cuticularized internal



TEXT FIG. 3. The apex of another mature seed of *Gnetum Gnemon*. The free upward flange (*Af*) is much smaller than in Text-fig. 2, and the papillate region (*P*) more clearly seen. The free part of the micropylar tube (*m.t.*) is shorter and comes to the surface of the outermost covering (*C*). *Bf* and *G* as in Fig. 2 D).

epidermis. A short way down (Photo 8, Pl. I) the free portion of the upwardly projecting flange is cut (see *Af*, Text-figs. 2 and 3), its inner edge fringed by crushed rows of cells with corky walls; growth from a cork cambium has occurred, cutting off dead and empty cork-cells over the exposed free region of the flange, and thus protecting the delicate tissues beneath. Photo 9, Pl. I, is from a section below the free flange, and shows the base of the cork cambium at the junction of the micropylar tube and the flange. The lumen of the tube is narrowed by the subdivision of the epidermal layer, while the outer surface of the proliferated wall is fused with the outer covering, and the boundary between them (*e*, Photo 9) is not readily distinguishable; in Photos 10 and 11 the inner epidermis of the outer covering is clearly marked, though the surface of the tube, as a consequence of the growth in thickness of its wall (now composed of 20 or more layers of cells), is firmly pressed against the outer covering and is fused with it. From here downwards to the bottom of the flange the limits of the two are often quite indistinguishable, though at intervals irregular cracks appear, filled with a mucilaginous substance. In Photo 10, Pl. I, the lumen of the micropylar tube has entirely disappeared, though its original position is still marked by two or more thick-walled cells which used to form part of its lining. This solid portion of the tube, sometimes, but not always, having a little strand of thick-walled cells in the centre, is continuous for some distance (see Text-figs. 2 D and 3).

About two-thirds of the way down the wide region of the tube the strand of thick-walled cells becomes more prominent, and an opening reappears among them filled, however, by a solid mass of large cells with their walls heavily cuticularized, irregularly orientated, and looking like thyloses or papillae cut at intervals (Photo 11, Pl. I). These are, of course, the papillae described before,¹ which grew out from the epidermal cells of the lining. The wall outside the solid mass is composed of fewer layers, so that it would appear that there has been much less activity of the outer layers of the tube wall; as a consequence, the lumen in this region has not been closed by external pressure but by the ingrowth of papillae.

The papillate region is very short; the papillae suddenly become torn and few in number, and the whole middle of the tube next disappears, having, as was seen in the longitudinal section, been broken across and torn out. In Photo 12 there is consequently a large open, often mucilaginous cavity (*G*), irregularly lined by torn cells and bounded by a few layers of cells firmly fused to the outer covering.

Photo 13, Pl. I,² passes through the sudden enlargement of the outer layers of the micropylar tube to form the downwardly directed flange (*Bf*) which in the young seed projects over the tip of the middle covering

¹ p. 7.

² N.B. Photos 13 and 14 are on a smaller scale than the others of this series.

(see, Text-figs. 1 and 2 B). A smaller lateral irregular gap (*g*) is seen in the flange, probably similar to that seen in Text-figs. 2 D and 3. It seems likely that these gaps represent the places into which the middle covering originally projected.

Owing to the very considerable growth and stretching of the apex of the seed between the stage 8 mm. long and these mature seeds, not only is the tip of the middle covering no longer buried in the hairs of the flange, but the flange has been carried so far upwards that there is a considerable interval during which sections pass through, first the outer covering only, and then the outer covering enclosing the torn-out, beak-like portion of the micropylar tube.

Cross-sections through the top of this projecting portion of the micropylar tube show its irregular torn surface and a wall of three or four layers of cells; the lumen is closed by the ingrowth of the epidermal cells. Lower down, its outer surface is smooth, bounded by a regular epidermis. All the cell-walls are heavily lignified, the projecting part of the tube being thus a solid little 'beak'.

When the series of cross-sections reach the parenchymatous tip of the middle covering it is found to be clasping firmly the base of the micropylar tube, which is still strongly lignified throughout, and is still closed. Photo 14, Pl. I, shows the micropylar tube a few sections lower down, with a slit-like opening, surrounded by the middle covering, which now has its inner layers heavily lignified and its outer parenchymatous tissue widely expanded.

Below this level the thick-walled inner layer of the middle covering persists to the base of the ovule, but the innermost covering becomes thin-walled and is seen in section as a thin strip of tissue free from the middle covering, fusing, about a third of the way down the seed, with the nucellus (see Text-fig. 2 C).

The structure of the outermost covering is similar to that in *G. africanum*, which is described in more detail in a former paper.¹ The middle covering is much as in the ovules 8 mm. long, except that the inner stony layer is more heavily lignified.

Two abnormal ovules. There were two curious features about the two ovules 8 mm. long. They were both flattened on one side, throughout the main body of the seed, being roughly triangular in form, like the seeds of *Ephedra*.

Also just inside the base of the inner covering there arose from the nucellus a ring of tissue, which was more developed in one ovule than in the other, but in both extended freely for some distance upwards like a rudiment of a fourth covering. A somewhat similar development, arising, however, between the outer and inner integument, has been

¹ Thoday, 1911.

recorded in an earlier paper on an abnormal ovule of *Welwitschia*,¹ and Pearson² described a fourth covering in the fertile ovule found at the base of a male spike in *G. africanum*.

G. funiculare.

The material of *G. funiculare*, kindly sent me from Buitenzorg, was not very well preserved, and has therefore not been investigated in great detail.

The small ovule. In the small ovules, up to about 5 mm. long, the three coverings are all free from one another. The outer one is already very fibrous. The upper portion of the micropylar tube had a very heavily cuticularized lining, like that of *Ephedra*,³ and contained a mass of hardened mucilage. Lower down, just above the tip of the middle covering, the lining of the tube ceases to be cuticularized, and the regularity of its cells is disturbed by a tendency to grow out into papillae. There seems to have been no increase in thickness in the wall of the tube, which consists of about the same number of layers, 5-7, throughout, and at this stage there is no flange. Some of the larger aborted ovules showed a few large irregular hairs growing out from the surface of the micropylar tube to form the flange.

From just above to some distance below the tip of the middle covering the micropyle is closed by the subdivided papillae, which form a solid rod of remarkably definite appearance, even more sharply defined than in the papillate region in *G. Gnemon*.⁴

The tip of the middle covering expands at the shoulders of the seed, but at this stage contains very little lignified tissue. Below the shoulders there is a layer of somewhat thickened horizontally running fibres. In the lower portion of the inner integument, below the micropylar tube, there are a large number of wide and strongly lignified fibres, which die out at the level of fusion with the nucellus.

The seeds. In the large seeds, about 2 cm. long, the outer covering is still more fibrous, there being two distinct zones of fibres, an inner and an outer, with thin-walled tissue between. At the apex the micropylar tube is closely approximated to the outer covering, and it appears to be fused with it for a short distance. The fused portion of the tube is, however, very short; it becomes free from the outer covering almost at once; there is a small flange, composed of a very few gigantic and very strongly lignified hairs or papillae, some above the fused region upwardly directed, some projecting downwards over the tip of the middle covering; they more or less fill up the chink between the top of the middle covering and the outer covering. The

¹ Sykes, 1910, Fig. 12, p. 199.

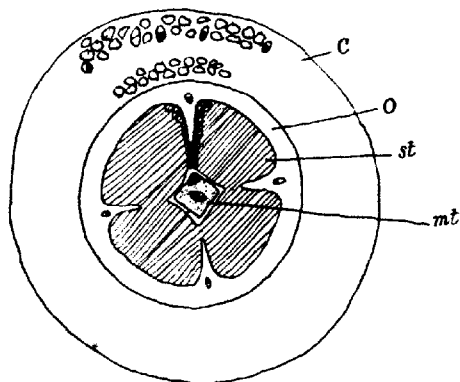
² Thoday and Erridge, 1912, p. 964.

³ Pearson, 1915, p. 322.

⁴ Photo 11, Pl. I.

tube is closed throughout this narrow region by the ingrowth of fairly regular epidermal cells with strongly lignified walls.

The middle covering is much more differentiated, and except just at its apex is now fused throughout with the outer covering, as in *G. scandens*.¹ In its expanded portions there are four large masses of lignified



TEXT-FIG. 4. *G. funiculare*. Cross-section of apex of seed, showing two bands of fibres in outer covering (c), 4-lobed stony layer (st), of middle covering (o), and lignified micropylar tube (mt).

fibres (Text-fig. 4) which, lower down, pass into a heavily lignified layer composed of numerous horizontal fibres and a sort of indefinite palisade layer of fibres elongated at right angles to these; the outer layers are parenchymatous.

The fibres in the inner integument have not much, if at all, increased in number, but the outer covering is very fibrous in this seed, not succulent as in *G. Gnemon*.

Comparative Summary of the Seeds of Four Species of Gnetum.

The seeds of *G. Gnemon* are by far the most interesting of the four species I have seen, because of the complex arrangement for the closure of the micropyle. In the mature seed the canal of the micropylar tube is not only closed by the ingrowth of the epidermal cells, and by pressure due to the great increase in thickness of its wall, but the tube itself is also firmly fused to the outer covering. An upwardly directed flange, covered with cork which protects its delicate tissues, and a downwardly directed flange also fused to the outer covering, complete the solid stopper-like arrangement. Just above this lower flange the micropylar tube is broken across, and the papillate cells, which in the earlier stages fill up the cavity of the lower part

¹ Thoday, 1911, p. 1118.

of the tube, where the wall has not increased so much in thickness, are mostly drawn out with the broken base of the tube. Thus the lower end of the tube is now found projecting as a solid, hard, lignified beak from the top of the middle covering.

Other characteristics of *G. Gnemon* are the angular outline of the middle covering, and the freedom of the two outer coverings from each other except at the apex of the seed.

In the seeds of *G. africanum* the micropylar tube is also closed by the ingrowth and subdivision of the epidermal cells lining the tube, and there is also a small flange which projects over the apex of the middle covering, but is not, unless at a later stage than I have seen, fused on to the outer covering. The micropylar tube has no papillate region, and is nowhere torn across. The middle covering in this seed is of highly complex structure. It is free from the outer covering.

In *G. scandens* and *G. funiculare*, which closely resemble one another, the two outer coverings are fused. The flange projecting over the tip of the middle covering is very slightly developed, consisting in *G. funiculare* of a few peculiarly large lignified hairs only. The tube in *G. scandens* is closed by ingrowth and lignification of the epidermal cells, and the concurrent growth in thickness of its whole wall. In *G. funiculare* the wall of the tube does not increase in thickness; consequently there is no pressure to assist in the closing of the lumen, and the ingrowing epidermal cells are looser and more irregular, like the papillate region in *G. Gnemon*. In *G. scandens* the middle covering, and in *G. funiculare* the outer and inner also, contain many strongly lignified fibres.

These four species thus show very different degrees of efficiency in the closure of the micropyle. In *G. funiculare* and *G. scandens* there is no gap between the two outer coverings of the ovule to fill, for these are fused in the mature seed, so there is no need of an elaborate stopper, and the small flange of hairs growing out of the micropylar tube and adhering to the tip of the middle covering is sufficient. In *G. africanum* and *G. Gnemon* the outermost covering is free from the middle, but in the former species the chink between the two is not completely closed, the stopper being well developed and projecting over the middle covering, but not being adherent to the outermost covering. In *G. Gnemon* the closing arrangement is complete, and the adherence of the stopper to the outermost covering has resulted in the breaking of the tube and the separation of the stopper from the micropylar beak.

Comparison with the Bennettitales.

From time to time comparisons have been made between the Gnetales and the Bennettitales; recently both Miss Berridge and I have drawn

attention to the resemblances in their seeds.¹ If there is any force in this comparison, it is certainly augmented by the structure of the mature seed of *G. Gnemon*.

Although it is not desirable to draw from these resemblances any conclusions as to affinity, it is at least possible that the more easily investigated modern seeds may throw some light on the fossils, and it may well be that common ancestors are responsible for some of the similar features. The remarkable growth-changes now described in the development of the seeds of *Gnetums* may help us to interpret the differing arrangements described in the various Bennettitalean seeds, and it is at least conceivable that these differing arrangements are to be ascribed to the varying stages of maturity at which the seeds had arrived.

A detailed comparison between some of the Bennettitalean seeds described up to that time and the seeds of *Gnetum* was made in a former paper,² and the recent descriptions of *Cycadeoidea* by Wieland and Seward's³ account of the small ovules of *Williamsonia scotica*⁴ seem to corroborate these early attempts.

In the small ovules of *W. scotica* the apex of the nucellus is composed of radiating rows of cells, and in some cases there is no sign of any disintegration and no pollen-chamber.⁵ In other cases there appears to be a small space at the apex, the sides of which extend upwards as a 'shrivelled beak-like prolongation',⁶ suggestive of the pollen-chamber of *Gnetum*, with its thickened and indurated walls, which in longitudinal section are seen projecting upwards like a minute beak. The seeds were not sufficiently well preserved, and probably too immature⁷ for much differentiation in the integument. Originating near the base of the seed, Seward found in longitudinal section 'two strips of indistinct, thick-walled and short cells which have no epidermis and are almost certainly portions of a tissue which was originally broader. At a higher level the short cells become rather larger and much longer',⁸ and are better preserved. These loose strips of tissue may represent the thickened fibrous layer of an integument like that of *Gnetum*, the rest having failed of preservation. At a higher level these strips approach the nucellus and join with it: this level would correspond with that of the origin of the inner integument in *Gnetum*, and, as in *Gnetum*, an expanded tissue is found over the shoulders of the seed, composed of thickened and radially elongated cells limited by a well-defined and characteristic epidermis. This epidermis ceases above the shoulders of the seed, just at the level where the middle covering ceases and we find the

¹ Sykes, 1912, p. 217; Berridge, 1911, p. 140; Thoday (Sykes), 1911, pp. 1125-30; Thoday and Berridge, 1912, pp. 978-9.

² Thoday, M. G., 1911, pp. 1125-30.

³ Seward, 1912.

⁴ Ibid., Fig. 18, Pl. II.

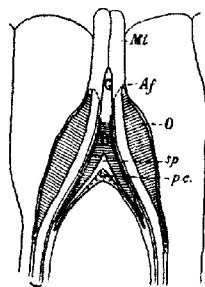
⁵ Ibid., pp. 110-11.

⁶ Wieland, 1912, Fig. 2.

⁷ Ibid., Fig. 3 D, p. 111.

⁸ Seward, 1912, p. 115.

expanded flange of the inner covering in *Gnetum*. The micropyle is widely open at the apex, but lower down, where the canal is probably slit-like, it may, if the comparison with *Gnetum* be correct, be already partly closed.



TEXT-FIG. 5. Apex of seed of *B. Morierii* (from Lignier, 1894, relettered for purposes of comparison). *Mi* = micropylar tube with projecting spurs (*Af* comparable with stopper and downward flange in *Gnetum*, projecting over tip of integument (*O*). (The dotted line at the base of the flange is inserted.) *G* = space between stopper and beak (*v*) described by Lignier as the canal of the micropylar tube; *n* = Lignier's nucellar beak, compared here with the beak formed by the broken-off base of the micropylar tube in *Gnetum*; *sp* = space between beak and nucellus called by Lignier the pollen-chamber; *p.c.* = Lignier's 'corpuscular mass' in the apex of the nucellus comparable in position with the pollen-chamber in *Gnetum*.

The earlier descriptions of *Cycadeoidea* made possible only a rough comparison with *Bennettites* and the mature seed of *Gnetum*, but the later described mature seeds of *C. turrita*¹ and *C. Dartoni*² make the resemblances much clearer. In these seeds the outer layer of the integument is 'palisaded' throughout, but most strongly at the shoulders of the seed, where it is expanded into five or six wings, like *B. Morierii*. At the top of these expanded shoulders there is a break, beyond which begins the micropylar tube proper; the stony layer of the integument is not prolonged over the micropylar tube as was at first erroneously thought.³ Thus the whole arrangement is quite comparable with that in *G. africanum*;⁴ the micropylar tube is even similarly filled with thin-walled tissue, and appears to be a separate organ from the integument which forms the shoulders of the seed.

There is no nucellar beak described or figured in these seeds; as they are at a stage when the embryo is well developed, it is not surprising that the relations of the micropylar tube to the nucellus, &c., are not well defined.

Lignier's description of *Bennettites Morierii*,⁵ also a seed with fully developed embryo, has up till now not been comparable with the other seeds, either of the *Bennettitales* or of *Gnetum*. In my earlier paper on *Gnetum*,⁶ I compared the detailed structure of its winged integument with that of *Gnetum* and *Ephedra*,⁷ but it was obvious that the relations of the nucellus with the micropylar tube and integument in *Bennettites* were very confusing. The suggestions then made are strengthened by the current investigation of *G. Gnemon*; the remarkable arrangement found at the apex of that seed, exemplified in Text-fig. 2, resulting from complicated growth-changes reminds one most forcibly of Lignier's figures

¹ Wieland, 1911, Fig. 15 c; 1912, Pl. VI, Fig. 11. He compares this seed to *Gnetum*, but his figure of the 'leafy' seed of *Gnetum* seems to be based on a misconception.

² Wieland, 1916, p. 133.

³ Ibid., 1912, Pl. VI, Fig. 6.

⁴ Ibid.; see description of Fig. 11.

⁵ Lignier, 1894.

⁶ Thoday, 1911.

of the seed in *B. Morierii* (see Text-fig. 5), the freely projecting beak-like base of the micropylar tube corresponding to his 'nucellar beak', and the space within it to his 'pollen-chamber';¹ the small lysigenous space described by him at the apex of the nucellus, and stated to have been once seen to contain objects which resembled pollen grains,² corresponds in position to the pollen-chamber in the young seeds of *Gnetum*. Lignier thought the integument and micropylar tube were continuous, though there was no detailed resemblance between their respective cell organization; and it may well be that the micropylar tube was here, as in *Gnetum*, fastened by a flange to the top of the outer integument,³ while the projecting 'beak' is really the broken base of the same micropylar tube.

Miss Berridge, while comparing the seed of *G. Gnemon* to *B. Morierii*, suggested that the nucellar beak in the latter is comparable to the closing tissue of the micropylar tube in *G. Gnemon*; Lignier (1911), in reply, draws attention to the massive structure of the nucellar beak in *B. Morierii*, which he says is 'totalement indépendant du tube micopylaire', while the tissue closing the micropyle is produced 'au-dessus du sommet nucellaire', that is to say, there is a space between the stopper, i. e. the closed apex of the micropylar tube, and the beak; there is just such a space now described in the mature seed of *G. Gnemon*, between the stopper and the broken-off base of the micropylar tube, the whole of which broken-off base (and not the closing tissue only) I am here comparing with the so-called 'nucellar beak' in *B. Morierii*.

SUMMARY.

This paper describes the series of changes which occur in the development of the seed in *G. Gnemon*. The young seed has three coverings, the inner of which projects freely upwards as the micropylar tube; by a series of growth-changes, the canal of the micropylar tube becomes closed and its apical region broken off from the base.

This apical region then forms a sort of 'stopper' firmly fused by a flange on to the outer covering. By the growth of the outer covering the stopper is carried up away from the lower part of the tube; a considerable distance is thus established between the stopper and the top of the middle covering, over which the flange originally projected.

The broken-off basal part of the tube then projects as a sort of 'beak' through the opening in the top of the middle covering; this beak is solid

¹ Thoday, 1911, p. 1128.

² Berridge, 1911; Thoday, 1911. In some seeds of *G. Gnemon*, Berridge, Fig. 1, and my two ovules 8 mm. long, the lower part of the micropylar tube below the closed region is lined by a ragged torn layer of cells very suggestive of Lignier's 'lysigenous pollen-chamber'.

³ Since the above was written Wieland (1916) has discussed Miss Berridge's and my earlier suggestions, and I am quite of his opinion that the above resemblances require further elucidation and comparison with the seeds.

and strongly lignified down to the level of the shoulders of the seed. When the beak or basal part of the tube is broken off from the upper part, some of the closing tissue of the stopper is generally dragged out, leaving a cavity in the bottom of the stopper.

An attempt is made to compare more closely than before the series of seeds belonging to the different species of *Gnetum* with some of those of the Bennettitales. *Williamsonia scotica*, with its simple plan, is compared with a young seed of *Gnetum*, and new light seems to be thrown on the complex structure of *Cycadeoidea* and *Bennettites Morierii* by the mature seed of *Gnetum Gneomon*.

The greater part of this work was done in the Manchester University Botanical Laboratory. My thanks are due to my husband, Professor D. Thoday, for his assistance in the preparation of the text-figures.

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DESCRIPTION OF FIGURES IN PLATE I.

Illustrating Mrs. M. G. Thoday's paper on Anatomy of the Ovule and Seed in *Gnetum Gnemon*, with Notes on *Gnetum funiculare*.

G. Gnemon.

∇ C = outermost covering; O = outer integument; I = inner integument; N = nucellus.

Figs. 1-6. Series of transverse sections through young ovule.

Fig. 1. Just below withered apex, showing open tube.

Fig. 2. Beginning of upwardly directed flange, which is seen on right free from the tube wall, but on the left is in continuation with it. f = flange; p = papillate outgrowth to form flange.

Fig. 3. Shows the ingrowth of the epidermal cells of the micropylar canal and the proliferated outer epidermis of the tube.

Fig. 4. Section through the base of the widened region of the tube, just about the level of the top of the middle covering. x = group of cells projecting from the tip of the middle covering among the hairs of the flange.

Fig. 5. Passes through the middle and inner coverings and shows the lumen of the micropylar tube still nearly closed.

Fig. 6. A little lower; lumen widened to a triangular cavity.

Figs. 7-14. Series of transverse sections through large seed.

Fig. 7. Free apex of micropylar tube with cuticularized lining.

Fig. 8. Free apex of micropylar tube a little lower down, showing also top of upwardly directed flange with corky covering. e = limit between flange and outermost covering.

Fig. 9. Level of origin of free flange from wall of micropylar tube; lumen nearly closed. e = limit between walls of inner and outer coverings.

Fig. 10. Micropylar tube quite solid.

Fig. 11. Section through region in which the wall of the tube is fewer cells thick and cavity is filled up with papillate cells.

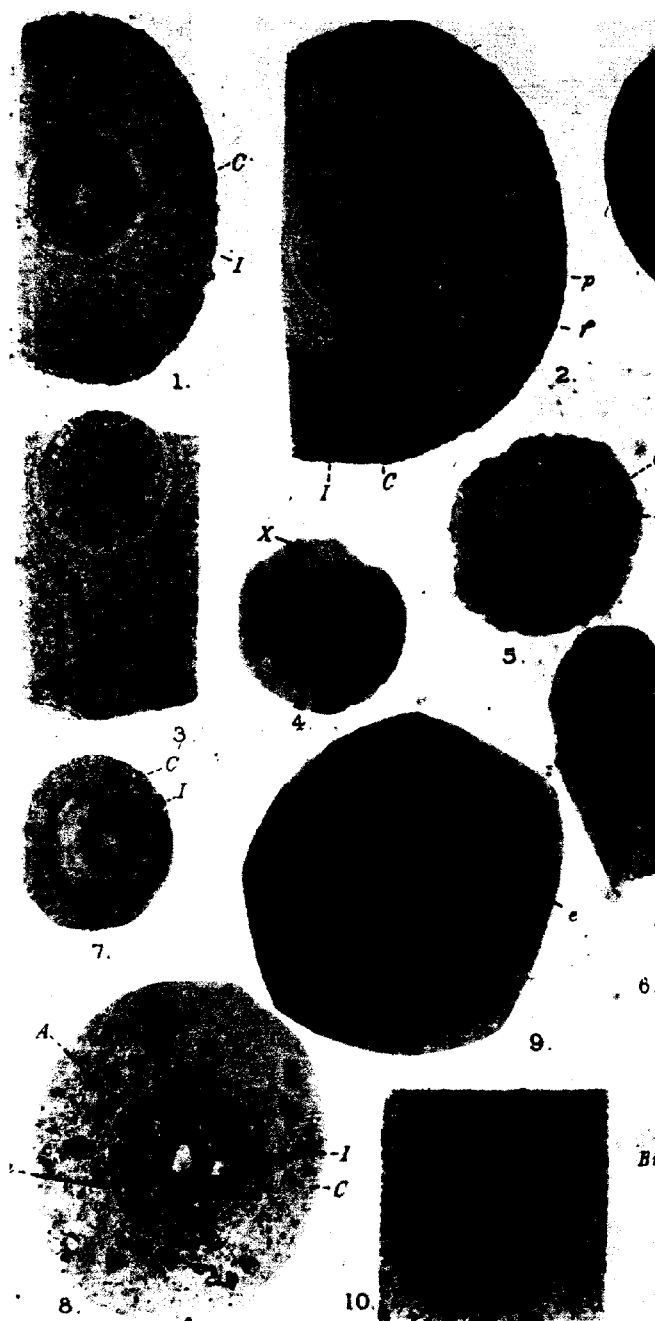
Fig. 12. The solid mass of papillae has been torn out, leaving a cavity with a rough surface (G).

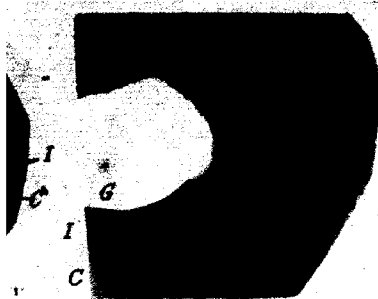
Fig. 13. Section through the base of the downwardly projecting flange; G is cavity of the tube from which the plug of cells has been torn. g = gap in projecting flange, magnified half as much as Figs. 7-13. (Between Fig. 13 and the tip of the middle covering the outer covering only is cut.)

Fig. 14. Near the tip of the middle covering, and shows the strongly lignified micropylar tube surrounded by the middle covering (ϕ), consisting of a stony inner layer (μ) and soft outer layer (σ). Radially expanded. Magnification as Fig. 13.

Fig. 15. Apex of young ovule: widely open micropylar canal with heavily cuticularized lining. μ = wall of inner covering (micropylar tube); σ = tip of middle covering (outer integument); ϕ = outer covering.

Fig. 16. Apex of rather larger ovule, showing remains of pollen-chamber ($\rho.c.$) and some divisions in inner epidermis of micropylar tube. N = nucellus.

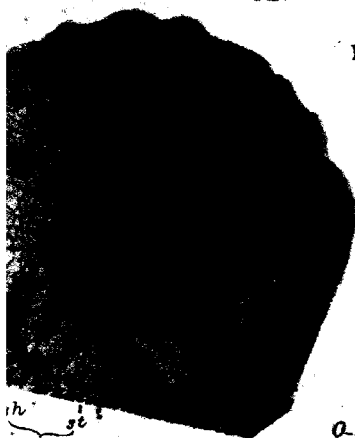




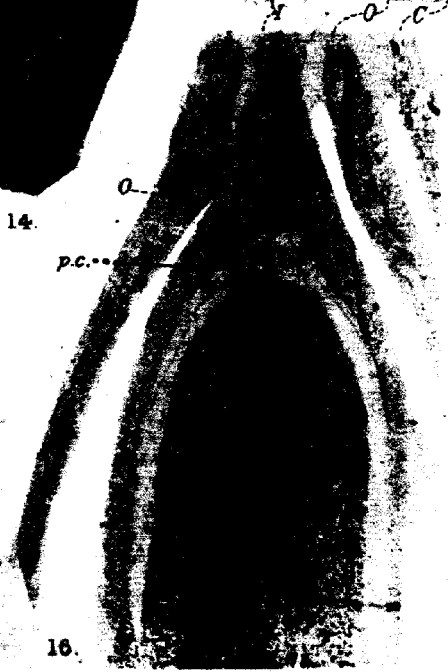
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15.



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13.

The Missing Link in Osmundites.

BY

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With Plate II and one Figure in the Text.

IN their classic series of memoirs on the fossil Osmundaceae, Kidston and Gwynne-Vaughan say (Part II, 1908, pp. 229, 230): '*the vascular anatomy of the Osmundaceae must be derived from a protostele with a solid central homogeneous xylem mass*,' and (in Part IV, 1910, p. 466): 'We regard the Osmundaceae, as a whole, as an ascending series of forms whose vascular system is to be derived from a primitive protostele with a solid homogeneous xylem.'

The missing link in the chain of practical evidence—that is to say, the actual species of *Osmundites*, possessing a solid stele and normal, simple Osmundaceous meristemes in the leaf-bases—was not known to them. The species about to be described from Australia fortunately shows this interesting conjunction of features; and, writing to me about photographs of the new specimen I sent to him, Dr. Robert Kidston, F.R.S., said: 'Your specimen seems to be the missing link we required.'

The specimen came to my hands, in the Geological Department of the British Museum of Natural History, some years ago, having been most kindly sent to us, with other material, by Dr. Walcott, Director of the National Museum, Melbourne, Australia. At that time the British Museum was planning, not only to describe the British Cretaceous fossil plants (see Stopes, 1913, 1915), but to prepare a complete comparative memoir on the Cretaceous plants of the whole world. As the war has rendered this project impracticable for many years to come, the present specimen seemed of sufficient interest to describe individually.

I am much indebted to Dr. Walcott, of Melbourne, for permission to cut the specimen, and to Dr. Smith Woodward, F.R.S., Keeper of the Geological Department, for his unfailing help and courtesy in arranging for the section-cutting and other facilities for utilizing the material in his unique department.

The specimen came with some others, all waterworn fossil woods, from Wollumbilla Creek, Queensland, described as 'probably Cretaceous'. The

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Osmundites consisted of a portion of a rhizome and surrounding leaf-bases, together appearing to have been roughly circular when complete, but of which only a portion like a sloping quarter cut out of a round cake was preserved. On the waterworn surface, part of which remains, the weathered and somewhat abraded leaf-bases are clearly visible. They vary, but measure roughly 4 mm. in long diameter of the surface face, representing cross-sections of the petioles. The specimen as a whole comprised a curved piece measuring about $3 \times 2.5 \times 1.5$ cm.

One portion of the specimen (V. 12640) and part of the slides (V. 12641 a, b, c, d, e, f, g, h, i, and j) are in the Geological Department, Natural History Museum, kindly presented by Dr. Walcott in exchange for the sections cut and sent to his museum; the other portion of the specimen and the other slides have been sent back to the National Museum, Melbourne, Australia. If desired by later workers, further sections could be obtained from the block in the British Museum, but this contains only leaf-bases and rootlets. A very short segment of the axial portion was preserved, and this was all cut up, yielding four slides and then running out of the specimen. Of these four sections the top and bottom (S 1 and S 4) each show good sections of the complete stele, surrounded by its single celled layer of 'sheath' cells. The two middle of these four sections each suffered partial demolition in the grinding down, so that in them the central axis, though in place, is incomplete.

GENERAL CHARACTER OF THE PLANT.

The new *Osmundites* consists of a slender central solid axis surrounded by a crowd of leaf-bases of which the meristele is a simple curved arc bent with the bow outwards and the horns inwards in the usual *Osmundaceous* way. There is nothing in the leaf-bases or the leaf-traces (so far as I can detect) which is not absolutely typical and characteristic of the genus. The presence of the sclerized patches within each curve of the meristele, the thickened oval round the meristele, and the lateral patches in the ground tissue on either side of it are comparable with those described by Kidston and Gwynne-Vaughan for *Osmundites* leaf-bases. In particular, the diagram of the leaf base given by them in their Part V, 1914, p. 478, Text-fig. 4, of '*Osmundites* sp. from Queensland', might almost have been drawn from my specimen. Compare my Text-fig with this. This feature can also be clearly seen in the middle of the Photo 6, Pl. II. Kidston's specimen, however, appeared to possess an ordinary *Osmundaceous* xylem in its main axis.

Interspersed with the leaf-bases in the usual way also, are numerous small diarch roots. The new specimen, therefore, is a quite typical and characteristic *Osmundites*, but for its remarkable and interesting main axis.

DETAILED DESCRIPTION OF SPECIMEN.

The *main axis* is a solid mass of wood. Photographs of the two good sections of this stele are shown on Pl. II, Photos 2 and 3; in Photos 4 and 5 are shown lower magnifications of the whole sections in which they lie. These make clear the relation and relative sizes of the leaf-bases surrounding it and of the main axis. The total diameter of the *xylem* of the main stele is roughly 1-mm., and the whole is irregularly circular with a slight tendency to a stellate shape. (This is of historic interest in comparison with the palaeozoic forms, such as *Zaleskya*, which Kidston and Gwynne-Vaughan link on to the group; see their memoirs quoted above.)

The whole of the central axial tissue appears to me to be tracheal, but about this I cannot be absolutely sure, owing to the absence of longitudinal sections. In the actual slides the solid nature of this central mass is very much more evident than in the photographs, particularly in the slide (V. 12641 *h*) from which Photo 3 is photographed. In this, when the high

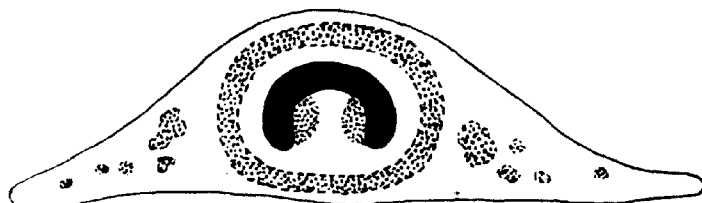


Diagram of leaf-base of *Osmundites Kidstoni*, sp. nov., showing the position of the metistele and sclerotic tissues: the latter are dotted. $\times 15$.

power is used with a much reduced orifice in the iris diaphragm, it reveals cell structure right across the space which, in the photograph, looks like a gap. The preservation of the cell-walls here is not optically sharply defined from the mineral matrix, while the cell-walls which show up clearly in the photographs have a strongly-marked contrast with the matrix, due to their black colour. So far as I can judge, this appears to be due to the chances of petrification, and not to any inherent differences in the natures of the cells. This interpretation is confirmed by the presence of a single cell or two which has one half of its walls black and strong, and the other half faint and translucent, and therefore, in the photograph, invisible.

Whether all the central cells were tracheides, or whether dispersed among them were some parenchyma cells, absence of longitudinal sections of this axis makes it impossible to say with certainty, but the appearance in transverse section shows a certain amount of the pitting, and there is no conclusive evidence against the view that the mass consisted almost entirely of tracheides.

The *protoxylems* are not clearly evident, but seem to lie somewhat

within each point of the seven-horned central mass. It is much to be hoped that further specimens of this interesting species may come to light and yield longitudinal sections to settle this and other points about the stele structure.

When one mentally subtracts the seven bays of *secondary* tissue, the star-like form of the primary wood becomes very evident, as is seen clearly in Photo 3. This is so remarkably like the central axial tissue of some of the palaeozoic Botryopterideae that one feels that Kidston and Gwynne-Vaughan's statement that they believe the Osmundaceous and the Botryopteridean series to have had a common ancestor (Part IV, p. 468) has, in this new plant, a strong piece of confirmatory evidence. They say: 'An Osmundaceous stele with a continuous ring of xylem and a mixed pith corresponding exactly to that of *Zygopteris Grayi* has not yet been found; if it did exist, the difference between it and the stele of *Zygopteris Grayi* would lie mainly in the star-like outline of the latter.' The present species has a star-like outline much like the latter. But it appears to be more primitive in having apparently a *solid* xylem and not a mixed pith. The presence of secondary tissue is, of course, a very unusual feature in the ferns of any age, and may, particularly when the primary wood is small, be considered a primitive feature.

The *secondary wood* forms seven compact bays which, together with the somewhat stellate primary wood, complete the roughly spherical shape of the stele (see Photo 3). The secondary and primary tracheides are not sharply distinguished, and, where they are adjacent, it is hard to say which is which. In the outward direction, however, they appear in fairly definite radial series, with a maximum of about seven tracheides in each radius. The greatest diameter of these secondary tracheides is somewhat less ($1.5-2\mu$) than the central primary elements of the wood, which vary between 2μ and 3μ .

The pitting of these tracheides also, so far as can be judged from hints revealed in the transverse sections, seems to be that usual in the Osmundaceae. The phloem and outer tissues are not preserved, and the space they must have occupied is filled with crystallized matrix. Surrounding the stele, and evidently outside the phloem, is a layer of large strongly-marked cells. These cells appear very comparable with those described as endodermis in *Osmundites Carnieri* (see Kidston and Gwynne-Vaughan, 1914. Pl. XLIV, Figs. 37 and 40). Outside this layer is again another space of crystallized matrix, in which must have lain the small, recently detached meristecles. At a short distance, about $2.5-3$ mm. (see Photos 4 and 5), the surrounding leaf bases are preserved; those nearer the axis are considerably crushed and distorted, probably owing to their delicacy. Those lying rather farther from the axis are less distorted, and some of the larger ones seem but little crushed (see Photo 1 and Photo 6).

The *leaf-base* is, as has already been mentioned, quite normal and typical for the Osmundaceae. An enlarged photo of the portions within the sclerized ring and the complete meristele are shown in Photo 6, and a diagram of the whole, showing the position of the *sclerotic nests*, is given in the Text-fig. In the detailed cellular structure of the leaf-bases of the new plant no feature meriting special description occurs. The xylem, phloem, and other tissues are apparently entirely usual for the leaf-bases of this family.

The *roots*, of which many are to be seen cut in a variety of directions in among the leaf-bases, are also typical for the family. Their stele is *diarch*, with a group of about a dozen tracheides and a well-marked sheath. See Photo 7, Pl. II. In longitudinal oblique sections, which are numerous, the tracheides appear to be provided with series of irregular rows of narrow pits. They appear to answer to the following description: 'The tracheae are of the typical Osmundaceous type; that is to say, the pits are actual perforations, and several vertical series of them occur on each wall' (Kidston and Gwynne-Vaughan, 1910, p. 456).

DIAGNOSIS.

As strict comparative diagnoses have not been given for the various species of the group, even in the Kidston and Gwynne-Vaughan Memoirs, it is more difficult than it inevitably need be to diagnose a new species from a single and incomplete specimen. Whether or not its small size is characteristic is hard to say; as the secondary wood of the axis was already forming, this may be a specific feature worth including in the diagnosis. The plant described above is named in honour of Dr. R. Kidston, F.R.S., of Stirling, who has done such unique work in Palaeobotany, and has specially interested himself in this group:

Osmundites Kidstoni, sp. nov.

General type of the vegetative body characteristic of the genus; leaf-bases and roots typical, but main axis a protostele with secondary wood. Roots diarch, adventitious among leaf-bases. Leaf-bases with a simple bow-shaped meristele, embracing a sclerenchyma patch in each curved end, and surrounded by a ring of sclerenchyma; irregular lateral sclerenchyma patches in the lateral wings of the leaf-base. Main axis apparently solid, roughly circular, made up of somewhat stellate, seven-rayed protostele filled in with bays of secondary wood. Tracheid diameter varying between $1.5\ \mu$ and $3\ \mu$. Stele surrounded by a sheath of dark cells. The whole plant very small, measuring less than 2 cm. from the centre of the stele to the largest and outermost leaf-base. The largest leaf-bases measure approximately only 3-4 mm. across the ring of circumstellar sclerenchyma.

Locality. Wollumbilla Creek, Queensland.

Horizon. 'Probably Cretaceous.'

Type (and only specimen). One wedge-shaped piece cut into slides: S 1, 2, 3, 4 include the main axis, of which no more remains; slides L 1, 2, and 3 cut longitudinally through the leaf-bases, and slides T 1 to T 12 cut transversely through the leaf-bases. Two pieces of the block remain, V. 12640 in the British Museum of Natural History, and a small piece returned to the National Museum, Melbourne. The slides are shared between the British Museum of Natural History (there numbered V. 12641 a to j) and the National Museum, Melbourne.

DISCUSSION.

The group has aroused so much interest and has already been so much discussed that it would be easy to enter into a lengthy statement of views about this new member. Its obviously interesting place in the *Osmundites* series will be apparent to all who know Kidston and Gwynne-Vaughan's Memoirs. It fits attractively into their theories, as I pointed out at the beginning, and its place is rendered all the more interesting from its comparatively late geological age. On *a priori* grounds, from the main axis it would have, probably, been judged to be of Early Mesozoic if not Coal Measure age, while the petioles are of Upper Mesozoic and recent type. Comparative accounts of the main genera associated with the group have already been made by others, so that I will confine myself to drawing attention to one plant now of interest which has not so far been much considered in comparison with the group, viz. *Botrychioxylon paradoxum*. Scott (1912). Secondary wood in ferns is rare; the two plants do not, at first sight, much resemble each other, owing to the very much greater extent of the secondary wood in *Botrychioxylon*, but if one imagines the secondary wood only partly formed, as can readily be done by shutting off the outer zones of it in the Fig. 7, Pl. XXXVIII, of Scott's paper, and comparing this with the Photo 3, Pl. II, a suggestive likeness can be seen which is rather more evident on comparing Scott's drawing, his Pl. XLI, Fig. 20. This comparison indicates one more link connecting the characteristics of the early Botryopterideae with primitive features in the Osmundaceae.

The web of forms representing the Botryopterideae (from some early representatives of which the consensus of opinion allows that the Osmundaceae were derived) shows a various shuffling, permutation, and combination of their features. It is interesting that we now have in this new species the actual combination of a solid somewhat stellate protostele together with secondary wood and an absolutely typical Osmundaceous leaf-base. Whatever its geological horizon, its phylogenetic value is, therefore, high, and it is still more intriguing if the form really lingered into the Cretaceous life of the Antipodes as this fossil makes probable.

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DESCRIPTION OF PLATE II.

Illustrating Dr. M. C. Stopes's paper on the Missing Link in *Osmundites*.

All photographs are enlargements of sections of *Osmundites Kidstoni*, sp. nov., and were taken by Mr. P. Dollman.

Photo 1. Transverse section showing the packed leaf-bases. The meristemes, sclerotic zones, and also adventitious roots can be seen. $\times 3$ (from Slide V. 12641c = T. 9).

Photo 2. Transverse section of the protostele of axis, showing the solid tissue. The apparent gaps and breaks are due to vagaries of fossilization which darkened some walls and left others translucent. Note the seven small bays of secondary wood, the large-celled sheath. $\times 80$ (from Slide V. 12641g = S. 1). This corresponds to A in Photo 5.

Photo 3. Transverse section of the same axis as above with an interval of two slides between. The seven bays of secondary wood and the somewhat stellate shape of the protostele show more clearly. $\times 80$ (from Slide V. 12641h = S. 4). This corresponds to A in Photo 4.

Photo 4. Transverse section of leaf-bases surrounding axis, A. The latter is that shown in Photo 3. $\times 3$.

Photo 5. Transverse section of leaf-bases surrounding axis, A. The latter is that shown in Photo 2. $\times 3$.

Photo 6. Single leaf-base enlarged, showing the simple curved meristeme embracing two patches of sclerenchyma and surrounded by the sclerotized ring. $\times 20$ (from Slide V. 12641b = T. 4).

Photo 7. Slightly oblique transverse section of adventitious rootlet, showing its diarch nature. $\times 20$ (from Slide V. 12641e = T. 6).



STOPES-OSMUNDITES.

Muth coll.

On the Pollination Mechanism of *Incarvillea* *Delavayi*, Franch.

BY

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With three Figures in the Text.

THE genus *Incarvillea* belongs to the Natural Order Bignoniaceae. The trumpet-shaped flowers are borne on a racemose inflorescence whose axis is about $1\frac{1}{2}$ to 2 feet high. Most of the flowers are placed near the top of the inflorescence: the few scattered lower down are arrested: they develop up to a certain stage, but grow no longer than about a third of an inch. The corolla tube of the ordinary flower is about two inches long, and directed slightly downwards (Fig. 1).

My attention was first drawn to these flowers because they possess a large sensitive stigma of the *Mimulus* type (Fig. 2, C, D, and E). A closer examination showed that there was a further point of interest in that the anthers had attached to them curious stiff, downwardly directed



FIG. 1. Side view of flower of *Incarvillea*
Delavayi (slightly reduced).

as the flower is naturally placed) prongs, and the opening of the anther-lobes and the consequent setting free of the loose pollen follows the manipulation of these. Each anther has two of these prolongations, one to each lobe (Fig. 2, E and F), and they are arranged so that one lobe of each anther must open and discharge some of its contents when the insect goes into the flower, and the other lobe is emptied of some of its contents on the insect coming out of the flower. The latter seems devoid of smell, at least in so far as it can be noticed by human senses, and the attractions that the flower has to offer are colour, conspicuous size, herding together at the top of the inflorescence axis, and a quantity of honey secreted by a large nectary situated round the base of the ovary. When the flower is quite open, the free portions widen

out so that the whole structure is about two and three-quarter inches long : the calyx tube is about three-quarters of an inch long. The width of the tube, at the place where the anthers are placed, is half an inch, and the width at the open end of the tube is about three-quarters of an inch (see Fig. 1). The tube is mostly yellow in colour, with purple spots, and the free portions are rose-purplish with purple spots.

The earlier opening flowers are lighter than those that mature later. A number of lines run down the corolla tube ; these may be interpreted as being honey guides, and two marked ridges run down the tube anteriorly. The lower petals are slightly larger than the upper ones, so that the corolla is asymmetrical. The flower does not completely open in dull and cold weather. (This condition probably does not occur in its native habitat in Yunnan.) The position of the flowers seems to be determined partly by gravity and partly by light, as in the *Narcissus*. No experiments were tried to test this statement, but most of the flowers were discovered to have so twisted their stalks (their origins are symmetrically placed on the axis) as to be facing away from the wall in the garden in which they were growing, and towards the south-west. Gravity probably has even more effect than this upon the development of the flower. In the young buds the stamens are similar and symmetrically arranged, and the axes of the anther-lobes of each anther are parallel with each other and with the fila-

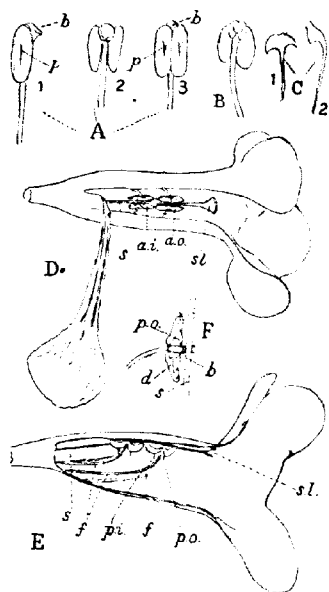


FIG. 2. A. Anther-lobes of longer stamen : 1, side view ; 2, back view ; 3, front view. $\times 2$. B. Back view of shorter stamen. $\times 2$. C. Young stigma : 1, front view ; 2, side view. D. Diagrammatic view of flower from below (with a slit in the side). E. Diagrammatic longitudinal section of flower. F. Diagram of anther showing the pad touching the style. $\times 1$. *f.* process ; *p.o.* outer process ; *p.i.* inner process ; *b.* 'buffer' or pad ; *a.i.* inner anther ; *a.o.* outer anther ; *f.* filament ; *s.* style ; *s.l.* stigmatic lobe ; *d.* slit in anther lobe.

ment (Fig. 2, A). As the flower grows older the anther-lobes on the shorter stamens become of dissimilar size (Fig. 2, B), and the lobes on all four stamens diverge until the axis of the two lobes of each stamen are in line and at right angles with the filament at the point of origin. This is comparable with the state of affairs in *Digitalis*. The stamens also change their positions relatively to the other parts of the flower. As the flowers grow older the filaments of the anthers twist round.

as has been described by Dr. Ogle in the case of the Foxglove (13), and as a result of this twisting the anthers take up their characteristic position, two on each side of the style (Fig. 2, D). Not only is this the case in *Incarvillea*, but the anthers are pressed closely against the style, a protuberant pad from the connective forming the actual point of contact and preventing the anther-lobes from touching (Fig. 2, F). We have, therefore, two pairs of opposed pads, touching the style, which act as buffers when the pollinating insect presses against the downwardly protruding anther-prongs. A few belated flowers opened at the top of the axis; they had but short stalks, and other, unopened buds were placed just above them. In these circumstances they were unable to twist round and take up their usual position, and it was found that the stamens did not change from the juvenile position. These latter changes are therefore probably under gravitational influence. Although these flowers never completely opened, both stigma and pollen matured, as both organs were capable of functioning; and the ovary, after fertilization, gave rise to a fruit.

The first prong of an anther that an insect would touch on coming into the flower is so arranged that a slit in the anther-lobe is placed above it and runs to the end of the lobe. The other thorny projection has the slit situated just below it (Fig. 2, E and F). In each case the surface of the lobe on either side of the slit is not a gently curved one, but is raised up in places and depressed in others. The significance of this configuration I hope soon to make clear. On the insect's back touching the first pair of prongs, one on each side of the style, the pressure is communicated to the pads and the two stamens and the style are kept rigidly in position. The sides of the slit or anther-cleft are pressed closely together and are therefore kept shut. When the insect touches the next pair of prongs, which will of course be on the same two stamens (and I imagine that a large insect like a humble-bee would touch both pair of prongs at the same time), not only are the pads brought into play, to cause the whole apparatus to remain in position, but as they are of some size and would not suffer much compression, in spite of their spongy nature, the sides of the anther-lobes are kept away from the style and do not touch it. This allows of considerable deformation taking place in the second pair of anther-lobes, and in consequence of this the slit opens and a certain amount of dry pollen is shed, passively, on the creature's back. The prongs, however, are very elastic and are attached each to a massive base composed, in great part, of thick-walled cells that resist a change of shape; therefore, on the insect passing, the projections are set free with a reverberation, which, if sufficient, causes a further and wider shedding of pollen, and, in any case, would redistribute the pollen-grains in the sac. It is seen, therefore, that the inequalities on the surface of the sac serve a double function. They prevent the pollen in the sac from being all shed at once, so allowing of several visits to the flower, and they also bring

about a wider shedding of the grains on to the insect's back than would be the case if the surface were smooth. It is possible that the creature makes several contacts with any one prong: this would result in several separate sheddings. On the insect passing to the next pair of stamens the same procedure takes place, so that on an insect entering the flower only the inner lobes of the anthers shed pollen on to it, and these probably do so on about the same spot. I am, of course, quite unable to be sure of this; the movement in or out of a flower can be imitated on the anthers by passing a finger along the points of the prong-like projections, but the exact details of pollination can be made out only in the natural habitat of the plant, and by an examination of the pollinating insect itself. When the insect makes its exit from the flower the reverse process takes place; only the outer lobes of the anthers open and pollen is shed from them. The figure of the anther given in Engler (14) shows a quite similar set of prongs, arranged as in *Incarvillea Delavayi* and *Incarvillea grandiflora* (a few flowers of this species were examined by me), and the connectival pad is also present, but the figure given of the flower of *Incarvillea Sinensis* shows the stamens emerging from the mouth of the corolla tube; if this is a normal flower of the plant, the pollination mechanism cannot be the same as in the two species here described. The flower, however, is drawn almost in a vertical position, and in two flowers of *I. Delavayi* that developed in a similar manner, owing to the flower-stalk being short and the other buds being crowded around it, the anthers were not able to take up their normal adult form. This may have been the case in the specimens figured in Engler. It would seem probable that neighbouring genera have similar mechanisms and would repay any observation of them. Schumann (14) describes and figures the lobes of *Amphicome* as having thorns and a leaf-like broadened connective. Unfortunately I have been unable, so far, to obtain specimens of this genus.

The possibility that some stimulus mechanism might be connected with pollen discharge in *Incarvillea* was not left untested. It was found that anthers which had been placed in alcohol for a period of at least a year were able to behave in a similar manner to the live ones. Sections of the anthers showed that they are provided with the usual fibrous cells; these, most probably, are concerned with the preliminary opening of the slit, and may also function in other ways: for example, in assisting in maintaining the necessary slight resistance to change of shape on the part of the anther. A microscopic examination of the stigmatic lobes of *Incarvillea* did not show the presence of any *Incarvillea* pollen, but there was plenty of foreign pollen, and an examination of this indicated that it was mostly that of the *Rhododendron*—some few plants of which grew in the garden near the experimental plants. As these are humble-bee flowers, and as I have seen these insects visiting the *Incarvilleas*, I have

but little doubt that they are responsible for the presence of the foreign pollen on the stigmas. That they do not seem to have pollinated the *Incarvillea* plants with their own pollen is no proof, however, that they are unable to set the mechanism in motion for the discharge of the pollen-grains, nor does it show that the grains are unable to adhere to their bodies. There were only three *Incarvillea* plants growing in my garden, and although I placed these close together, the chances of a bee's visiting two of these flowers, one after the other, were exceedingly small, because there were but a few flowers out at a time, probably never more than four. The stigmas are covered with papillate hairs that are doubtless used in pollen fixation. The flower is sufficiently preserved from self-pollination by the closure of the stigmatic lobes on stimulation. In the flower of *Thunbergia alata* (8, 11), which in mechanism comes closer to *Incarvillea* than any other plant known to me, it is curious to find that Darwin reports (3, 4) 'spontaneously self-fertilized fruits'. The stigmas of *Thunbergia* are not, as far as I am aware, sensitive; their position, however, and the method of shedding pollen in this plant suggest either that Darwin's plants were parthenogenetic or that insects did visit them and cause pollination, although they may not have been the insects that usually bring about the transference of pollen in this genus. The figure of the flower of *Thunbergia alata* copied from Hildebrand's paper and figured in Loew's 'Blutenbiologie' suggests that the anther-lobes are smooth and all the spines backwardly directed from the anthers. Lindau's figures (9) bear this out for *Thunbergia reticulata*, and it will be found that here the anther-lobes remain parallel, and the spines are so arranged that an insect only causes pollen to be shed on its emerging from the flower.

The flowers of *Incarvillea* had been found, in previous years, to be visited commonly by earwigs, and though it is not suggested that these creatures brought about the pollination in these cases, the plants undoubtedly bore fruits containing what looked like quite well-developed seeds. These seeds have not, however, germinated. This year, besides the earwigs, other visitors have been noticed, mostly a large garden ant—attracted by the honey—aphides, and an occasional humble-bee. I see no reason why the last named should not be able to bring about the pollination of these flowers, and, indeed, I should think that the setting of seed in the cases mentioned above was probably due to their agency. The humble-bee has about the right-sized body for the corolla tube, and also (I should imagine) the strength necessary to move the anther spines. I cannot, however, think how the pollen adheres to his body except one postulates that, as happens in other cases, a sticky secretion from the stigma is deposited on it as the bee passes into the flower. The stigmas I examined, however, were not sticky. The flowers, which grew in my garden and gave rise to fruit, had all been hand-pollinated; and all the flowers that had not been so treated

withered. The first few flowers to be pollinated by hand were not covered afterwards, but the last few were more carefully treated. They were unopened, but adult, flowers, and they were bagged after the pollination had been effected. Self-pollination and cross-pollination both gave rise to young fruits: these did not mature; this was not due to any deficiency on their part, but to a withering away of the inflorescence axis. I hope, in time, to overcome what is probably only a cultural difficulty and to get the seeds to ripen and to germinate.

POLLEN OF *INCARVILLEA*.

The pollen-grains (Fig. 3) resembled those of *Thunbergia alata* as figured by Goebel (6) in the 'Outlines of Classification and Special Mor-

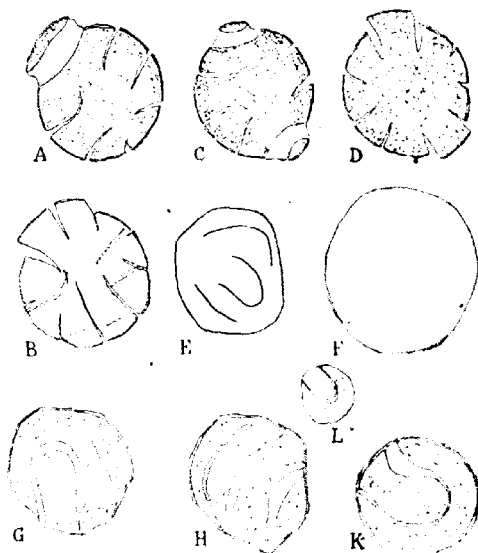


FIG. 3. Pollen-grains of *Incarvillea Delavayi*, A-F, and of *I. grandiflora*, G-L. All $\times 250$. A and B, same grains at upper and lower focus respectively, B with the slits seen in A dotted in. C and D, upper and lower views of the same grain. E, an air-dried grain. F, size of grain in 25% sugar solution. G and H, same grain at upper and lower focus. K, a similar grain. L, a small grain. (A, B, C, D after treatment with sulphuric acid.)

phology'. They were dry, and on placing them in water they were immediately ruptured; even in a mixture of equal portions of water and glycerine the majority of the grains were destroyed after a few minutes. The exine was cutinized and was resistant to the action of sulphuric acid; while the intine was dissolved in this reagent and gave the usual tests for cellulose. After such treatment, and occasionally before treatment, when mounted in strong sugar solutions, it could be seen that the outer wall was covered with

very minute papillations. The exine had long slits very much like those of *Thunbergia* (Fig. 3, A, B, C, D), but while in this genus the slits form one or two continuous lines, in *Incarvillea* the grooves only occasionally join together, so that we never get the appearance seen in Fig. 295—V, VII, and IV—of the 'Outlines' (Goebel) (6), the closest approximation being shown in my Fig. 3, C, where a few slits will be found to join, with the result that a portion of the outer wall opens out after the inner wall has been dissolved by sulphuric acid. An opportunity presented itself of examining a few withered flowers of *Incarvillea grandiflora*, in which plant the floral mechanism is quite similar to our species, the difference being in details, such as the longer flower stalk and the slightly different colour and marking. The pollen of *I. grandiflora* proved to be similar to that of *I. Delavayi*, but there were fewer slits and a very few small adult grains were to be observed amongst them (Fig. 3, G, H, K, L); the meaning of this heterospory is not clear to me. As there were two lengths of filament, pollen-grains from the anthers at different levels were examined in both species and closely compared as regards size and markings, especially as some such differences might have been expected, and in *Torenia*, for example, one pair of anther-lobes bears dry, the other pair moist, pollen; no differences, however, of any kind could be noticed in the case of *I. Delavayi*. Whether the different sized grains are commonly found in *I. grandiflora* or whether they are confined to the longer or shorter stamens, I am unable to state. In *Torenia* (2, 10), it will be remembered, only the dry pollen is immediately effective in pollination; the moist pollen having to be dried before it will bring about fertilization. In *Lagerstroemia indica* (4, 7) there are also two kinds of stamens—conspicuous ones, with moist pollen, visited by the insects and used as food, and inconspicuous ones containing dry pollen, used in pollination. Seemingly both kinds of pollen can in this case cause fertilization without any preliminary treatment. In *Incarvillea* the moist pollen need not be dried before it is effective. On exposure to air the pollen-grains immediately begin to lose water, get slightly smaller, and change shape from the spherical; the portions of exine between adjoining slits heaping themselves up (Fig. 3, E) (cf. figures of *Thunbergia* (5)), the water loss probably taking place mostly through the intine and the lips of the slit then approximating, and further loss causes a hinge-like movement along the crack. This approximation of the sides must considerably reduce the further loss by evaporation. Such drying would probably take place on the back of the insect carrying the pollen from one flower to another, though it is questionable whether the slight irregularity of surface so brought about is of any great importance for further fixation to the creature. The presence of slits and the high osmotic pressure of the contents, the evidence for the existence of which will be described below, I regard as adaptations to prevent drying.

Strasburger has (in his paper 'Ueber den Bau und das Wachsthum der Zellhäute' (15)) described shortly the development of the walls of the pollen-grains of *Thunbergia alata* and *T. reticulata*. I hope to study the development of the peculiar exine of the *Incarvilleas* at some future date.

All efforts to bring about the germination of the pollen-grains failed; 25 per cent. and 30 per cent. sugar solutions were tried, ordinary room temperatures and above room temperatures, up to 35° C., in the light and in the dark, and in some cases peptone was added to the sugar solution. Only the very first beginnings of a tube were noticed, and these were commoner at the higher temperatures, but did not persist in growing. At quite low temperatures, however, they were evidently able to germinate in the open air, on the stigma of the plant, as when they were placed there the ovaries began to swell in a few days' time and the fruit to ripen. I had but few fresh flowers to experiment with, and these had to be carefully conserved, so I could only make one preparation in sugar solution including a stigma, but even in these conditions the grains did not germinate properly. The moist-air method advocated recently by Anthony and Harlan (1) in the 'Journal of Agricultural Research' was also used, but with no favourable result. When placed in the sugar solution the grains at once increase in size (Fig. 3, F) markedly, and some few (when the 25 per cent. solution was used) burst open. The slits widen, and by next day those grains that show the beginnings of germination have an irregular bulge coming from the portion of the intine lying between the openings of the exine. The walls of these bulges soon thicken considerably and growth stops. The intine, both of grains showing signs of germination and those that show no such indications, are very much swollen, and I am of the opinion that it is this increase in size that stops the growth of the contents.

SUMMARY.

A mechanical method of pollen-discharge is described in *Incarvillea Delavayi*, a plant possessed of a sensitive stigma. The pollen of the plant is dry, and is similar in structure to that of *Thunbergia alata*. The high osmotic pressure of the contents and the approximation of the walls of the surface slits, after drying, are regarded as adaptations to prevent too great a loss of water in pollen transmission.

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Telephragmoxylon and the Origin of Wood Parenchyma.¹

BY

R. E. TORREY.

With Plate III and three Figures in the Text.

IN Jeffrey's 'Anatomy of Woody Plants', published in 1917, a theory of the origin of wood parenchyma is advanced. From a study of living woods, and particularly those of the Coniferae, the author was led to the conclusion that this important element originated from the so-called septate tracheide and that its first appearance was at the end of the annual ring.

While the book just mentioned was still in the press an interesting and further confirmation of the theory came to light through the discovery of a new wood from the Comanchean deposits of Texas. A certain histological feature was judged of sufficient importance to justify a new generic name—*Telephragmoxylon*.

The technical description of the wood, along with many others derived from Mesozoic and Tertiary deposits, has been embodied in a memoir which will appear later. The reason for taking the genus from its original setting for individual treatment may be explained by a quotation from a recent letter of Professor Jeffrey's: 'It seems to me extremely desirable that you should write a special article on *Telephragmoxylon*. The genus is so important and so interesting that it should be brought to attention in one of the general journals.'

It is commonly known that Texas is well favoured with immense deposits of Tertiary lignite which are of considerable economic importance. On the other hand, it is probably known only to geologists that in far more ancient deposits of the same state, namely, those of the Triassic and Lower Cretaceous or Comanchean, may be found scattered and very local masses and fragments of lignitized and partially silicified woods.

In the fall of 1917, while collecting near Weatherford, Texas, the writer came upon fragments of charred and flattened trunks of a wood which seems to be new to science and to afford a striking justification of the theory

¹ Contribution from the Laboratories of Plant Morphology of Harvard University.

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just mentioned: namely, that wood parenchyma arose by the septation of tracheides at the end of the annual ring. Two species of the wood have been recognized. The following description is based upon *T. brachyphylloides*.

From an examination of the microtome sections, the first and most obvious fact is that we are dealing with a wood belonging to the Araucarian complex which dominated the Mesozoic forest. The bordered pits on the tracheides are uniseriate and are commonly tightly pressed against one another, though on larger tracheides biseriate alternation is not uncommon. The pit mouths are elongate and oval.

The rays are manifestly Araucarian; in tangential aspect they show themselves as either uniseriate or biseriate. Their cells show a slight gummy inclusion. The lateral tracheide field (as seen radially) is impressed with few to ten small pits which have either oval or circular openings. These Araucarian features are well shown in Fig. 1, Pl. III. Traumatic vertical resin-canals are present—a fact very suggestive of the phylogeny of the Araucarians, but one with which we are not now concerned. They are shown in Fig. 2, Pl. III. The wood at this point is poorly preserved, but it came from the same blocks that have exhibited the feature which makes the wood distinctive.

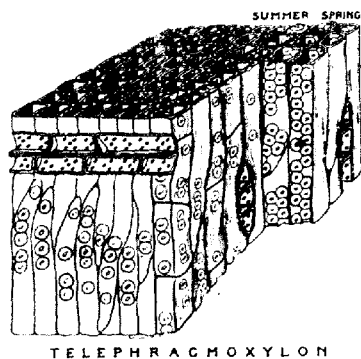
In sections inclined slightly from the true tangential plane towards the transverse, so that summer as well as spring wood may be included, there is manifest in many of the *terminal tracheides* of the annual ring successive and apparently double diaphragms which cross their lumina. These diaphragms are the end walls of short tracheides whence the middle lamella has disappeared. Each bears from one to three bordered pits. In other words, we are dealing with a condition of initial septation of the tracheides at the terminus of the annual ring: hence the generic name *Telephragmoxylon*. That these plates and their pits are very abundant and definite is evidenced by our microphotographs.

In Fig. 3, Pl. III, is seen a cross-section of the wood. Stretching obliquely across the field is a darker band of tracheides. The darker colour is due to the fact that a lucky section has cut at least five successive tracheides at such a point that their cross-diaphragms are visible. On one of these septa a pair of bordered pits is seen, and evidence of the same is visible on three others.

In Fig. 4, Pl. III, of a tangential section, an oblique band of septated tracheides runs across the field. Even with the low magnification employed it is evident that bordered pits are present in the septa. Fig. 5, Pl. III, shows a detail of these same tracheides under higher magnification.

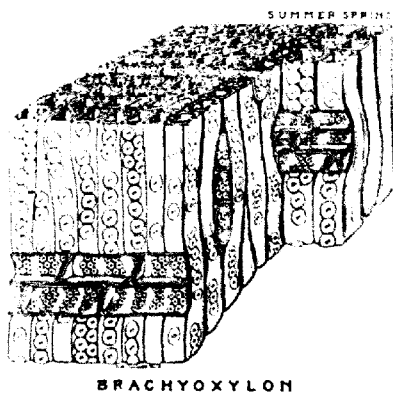
In Text-fig. 1 is shown a stereogram reconstruction of a small block of *Telephragmoxylon* wood. In Text-fig. 2 we have a similar figure of the allied genus *Brachyoxylon*. The essential difference is obvious.

To those who are familiar with the modern theory of the origin of wood parenchyma it is at once clear that we have here direct testimony from a fossil form as to the correctness of that view. It has been shown that in *Spruce* wood, for example, elements are laid down at the end of the



TELEPHRAGMOXYLON

TEXT-FIG. 1.



BRACHYOXYLON

TEXT-FIG. 2.

TEXT-FIG. 3. *Pt. ca.* Origin of parenchyma.

growing season which at an early stage 'become transversely septate, and in the segments so set off the protoplasm sometimes persists (when a typical parenchymatous element of the wood is the result); at other times it disappears with the complete differentiation of the walls surrounding it (in the case of so-called short tracheides)'. Text-fig. 3 illustrates this condition in a diagrammatic manner, while Fig. 6, Pl. III, is introduced to show by an

actual photograph a cross-section of *Spruce* wood and the position of wood parenchyma among the summer tracheides. In *Picea*, then, the midmost point of the differentiation of long tracheides into parenchyma has been reached. Yet logic requires us to suppose that the initial step in the process was the septation of the long tracheide into short tracheides, all of which were essentially similar. Such a situation appears not to have been detected in living woods, but in the fossil form under discussion just that condition is strikingly manifest.

That it belongs to a different genetic series is of no consequence. We are here dealing with the origin of wood parenchyma, not with the origin of *Picea*, and we know that this element of the wood has originated several times and independently.

It has been suggested that fully developed wood parenchyma at the end of the ring serves as a storehouse for foods which may be directly available to the developing cambium in the succeeding spring; and that its differentiation was correlated with the progressive refrigeration which characterized the last part of the Mesozoic Period.

Without bringing in a teleological explanation, we may believe that the original tracheide septation, as exemplified by the genus discussed in this article, constituted a superior mechanism for facilitating the rapid, radial transference of water and food-materials to the cambium at the same critical period of growth.

It should be pointed out that a wood of this character has once before been discovered. In 1913 Miss Ruth Holden found, among the fragments of lignitic wood from the Jurassic deposits of Yorkshire, a specimen of *Brachyoxylon* which, to quote her own words, is 'unique in the possession of large numbers of septate tracheides at the beginning (?) of each annual ring. The significance of these cells it is difficult to infer. Whether they represent incipient parenchyma or are related to an injury it is impossible to say, but the latter supposition is rendered improbable by their appearance in several successive years and the lack of any twist in the grain which would indicate proximity to a wound.'

Hence we are fairly certain that wood of the genus *Telephragmoxylon* was growing in Europe in the period which preceded the one in which our own specimens have been discovered.

As to its taxonomic position, *Telephragmoxylon* is a member of the sub-tribe Brachyphylloideae of the tribe Araucarineae, family Pinaceae.

SUMMARY.

From a study of living coniferous woods the theory has been advanced that wood parenchyma arose by septation of long tracheides, and that its first position was among the terminal cells of the summer wood. Pro-

gressive differentiation and diffusion through the ring was intimately related to the progressive refrigeration of the later Mesozoic.

From the Comanchean deposits of Texas have recently been obtained two woods of Araucarian affinities which exhibit the incipient stages of tracheide septation demanded by the theory. These have been placed in a new genus, *Telephragmoxylon*.

In conclusion, the writer expresses his indebtedness to Professor E. C. Jeffrey for assistance in the photographic work and for permission to use Text-figs. 1, 2, and 3.

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EXPLANATION OF PLATE III.

Illustrating Mr. R. E. Torrey's paper on *Telephragmoxylon* and the Origin of Wood Parenchyma.

Fig. 1. Radial section of the wood of *Telephragmoxylon brachyphyloides*, showing the characteristic ray and tracheide pitting.

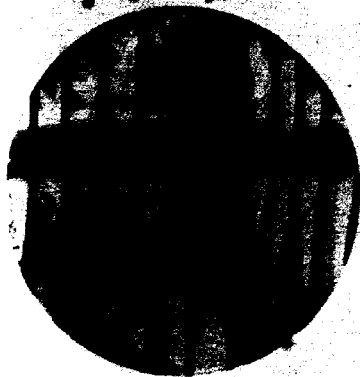
Fig. 2. Cross-section of the same, showing the Brachyoxylid traumatic resin canals.

Fig. 3. Cross-section of the same, showing the horizontal and pitted walls of the short tracheides.

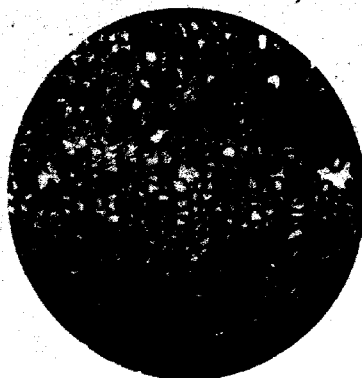
Fig. 4. Tangential section of the wood, exhibiting the septated tracheides at the end of the annual ring.

Fig. 5. Detail of the last under higher magnification.

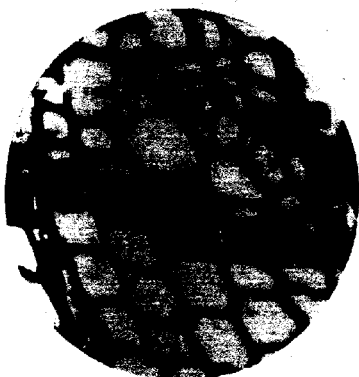
Fig. 6. Cross-section of the wood of *Picea sp.* to show the position of the wood parenchyma on the face of the summer wood.



1.



2.



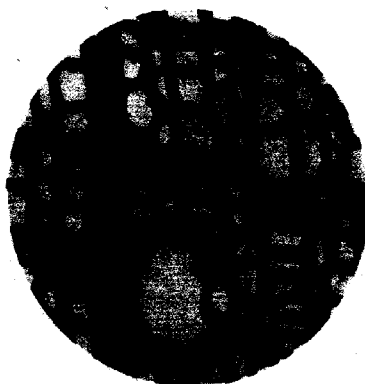
3.



4.



5.



6.

Some Observations on the Life-history of *Nectria galligena*, Bres.

BY

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With Plates IV and V.

INTRODUCTION.

THE present investigation was started with the object of finding, if possible, the most vulnerable stage in the life-history of *N. galligena* at which fungicides could best be applied to prevent the spread of the disease. This involved a complete study of the life-history of the fungus itself, and more especially the transition from the summer or conidial stage to the resting or perithecial stage. During the course of this part of the investigation a few hitherto unrecorded observations were made, which were thought to be of sufficient interest to allow of publication.

The description of the life-history is necessarily incomplete, owing to technical difficulties. The fungus is very small and difficult to deal with. The fruiting stages occur on dead or dying bark, and it was found impossible to fix and cut serial microtome sections thin enough to allow of accurate microscopical investigation. It was only after a medium had been elaborated, on which the fungus would complete all the known stages in its life-history in pure culture, that further progress could be made. The preparations on bark, however, served as controls for comparison with the growth in pure culture on artificial media, and the development of the life-cycle proved to be very much the same in both cases.

The fungus is a common parasite on apple and pear trees, giving rise to one of several forms of canker which occur on the stems and branches.

Nectria, under the name of *N. ditissima*, has been described by various workers on a number of other host plants besides apple and pear, such as copper beech, oak, hazel, ash, lime, and others; but it is questionable if these different investigators were really dealing with one and the same fungus. *Nectria* on beech was first described by Tulasne (31), in 1865, under the name of *N. ditissima*, and later Goethe (14-18) was able to show,

(Annals of Botany, Vol. XXXV. No. CXXXVII. January, 1922.)

by cross-inoculations, that copper beech could be infected by conidia of *N. ditissima*, Tul., isolated from canker on apple. He also showed that, although *N. ditissima* was generally considered to be a wound parasite, it was possible to infect uninjured shoots with conidia and ascospores, if the shoots were cut off and left without water. Under these conditions infection took place chiefly through the lenticels.

Since Tulasne, a considerable amount of work has been done on the fungus at various times by many different investigators, the most prominent being Willkomm (35), Hartig (20, 21), Goethe (14-18), Lapine (24), Aderhold (1, 2), Appel (3, 4), Wollenweber (3, 4, 36), Weese (33, 34), and others. Willkomm (35), in 1866, was the first to show that canker on beech trees was due to a fungoid parasite, but unfortunately his discovery led no further, as he did not definitely identify the fungus. He observed the conidial stage on beech and called it *Fusidium Candidum*. Hartig (21), took up the investigation in 1877, and showed that beech canker was mainly due to *N. ditissima*, Tul., although cankerous disorganization of bark could be caused by frost and insect injury. On the other hand, Weese (34), one of the more recent investigators, who has studied the Nectriaceae from the systematic point of view, is of the opinion that canker in fruit and timber trees is due to *N. galligena*, Bres., and not *N. ditissima*, Tul. (syn. *N. coccinea*), as held to be the cause by Hartig (20, 21), Goethe (14-18), and Aderhold (2), and that the two fungi are biologically and morphologically distinct. *Nectria galligena* causes definite cankers, whereas *N. ditissima*, Tul. (syn. *N. coccinea*), only breaks out of the bark and does not give rise to cankers. Weese (34) considers the most important characteristics which determine the variety are the formation of the perithecial wall and spore differences; that the presence of a stroma or subiculum is not of such systematic importance as was formerly held, and that one and the same fungus can occur, with or without a stroma, on the same piece of bark. Variations as to the presence or absence of stroma in Hypocreaceae have also been noted by Theissen (30), v. Hoehnel (23), and Wollenweber (36).

It is the opinion of the present author that the *Nectria* with which those workers were dealing—Hartig, Goethe, Aderhold, and others, who proved by inoculations that it gave rise to definite cankers on apple, beech, and other trees, and which they described under the name of *N. ditissima*, Tul.—must have been *N. galligena*, Bres., and that the slight differences between the two fungi were probably overlooked. Goethe had doubts as to whether the fungus with which he was concerned was really *N. ditissima*.

The fungus described in this communication was obtained from canker on apple, and agrees with the description of *N. galligena* given by Weese (33), except that the ascospores, obtained from dehiscing perithecia, and measured

fresh, proved to be rather larger than the dimensions given by Weese. *Nectria ditissima* has shorter ascospores.

Hartig (21) describes the three main stages in the life-history, namely, the microspore, the macrospore, and the perithecial stages, and also found, as did Tulasne (31), spermatogonia and spermatia on beech. The best early description of the fungus is given by Goethe (16), 1880, but both he and Lapine (24), 1892, failed to find spermatogonia on apple, in spite of careful search. During the present investigation spermatogonia or pycnidia were observed occasionally on apple bark affected with *Nectria*, but so far there is no conclusive evidence that the pycnidia observed belonged to *N. galligena*. This point will be more fully discussed later. Much of the work of the later investigators deals with the parasitism of the fungus and the effect of cultural and external conditions on the host and parasite, points which do not come within the scope of this communication.

In *N. galligena*, so far as is known to the author, no investigator has been able to find any definite nuclear fusions, either before the formation of the perithecium or within the tissues of the same. Nuclear fusions have been described within recent years in a number of Ascomycetes, including a few Pyrenomycetes, such as *Gnomonia erythrestoma* (6), *Polystigma rubrum* (5), *Aspergillus herbariorum* (13), &c., and it was thought possible that a more or less reduced stage might occur in *N. galligena*, which so far had been overlooked.

METHODS.

Portions of diseased bark and pieces of the medium on which pure cultures were growing were fixed in various fluids, with very variable results. All fixatives containing alcohol, such as acetic alcohol, formal alcohol, 70 per cent. alcohol, absolute alcohol, &c., were found to have a disturbing effect on the nuclei of the ascogonia and had to be abandoned, although the penetration was good.

The only fixative that proved to be of any use for the purpose of the investigation of nuclear detail was Flemming's weaker solution, and the time allowed varied according to the age of the perithecia to be fixed. Perithecia in the youngest stages on artificial media required from 4 to 6 hours' fixation, whereas perithecia on bark and older perithecia on artificial media required longer, and could be left on the fixative 24 hours without harm.

The results were very variable, in spite of the use of the air-pump. This is not surprising considering that tissues of extreme delicacy are enclosed within perithecial walls which vary in toughness according to age. In some cases 1 per cent. urea was added to Flemming's weaker solution to hasten penetration, but the results were disappointing, and latterly Flemming only was used throughout.

Some of the material, especially that on bark, was dehydrated in glycerine and some in alcohol, cleared in chloroform, embedded in 54° C. paraffin, and cut from 2 to 4 μ thick. The youngest stages were cut 2–3 μ .

It was found necessary, except for very young material, to use Henneguy's (22) gelatine-bichromate solution for mounting serial sections to prevent the sections washing off during the process of staining. The solution was prepared as follows: One gramme of gelatine was dissolved in 5,000 c.c. tap-water, to which a trace of bichromate of potash was added before use. The ribbons were placed on the slide, and the gelatine solution—which had been previously warmed in the 54° C. paraffin bath—was pipetted on to the slide. In this way the ribbon expanded rapidly without melting. Sometimes it was found necessary to warm the slide also, with the ribbon still floating on the gelatine solution. The superfluous liquid was then poured off and the slide blotted very gently and placed on several layers of blotting-paper on the top of the paraffin bath, to dry in the light for at least four hours. The bichromate did not seem to interfere with the staining as long as watery stains were used.

Heidenhain's iron-alum-haematoxylin gave by far the best results and was used throughout, counterstained with a saturated solution of lightgreen or erythrosin in clove oil.

MEDIA.

Conidia and ascospores of *N. galligena* will germinate well on a number of artificial media in common use in the laboratory and will develop considerable mycelial growth, but it was some time before a medium was found on which the fungus would go through all the stages of its life-history. The development is slow, and it takes at least 6–8 weeks, mostly longer, before the perithecial stage appears. Hence a medium had to be devised which would not dry out during that period. Platings proved to be useless on account of this tendency to dry out. Platings were made and the colonies transferred to slopes, or spores from pure culture sown direct on potato, &c.

The only media on which the fungus would develop perithecia were those which contained starch, or some derivative of starch, together with glycerine.

It is to Goethe (16) that we owe the observation that the starch disappears in the starch-containing cells of the cortex when attacked by *Nectria*. It was found that spores sown on potato only gave rise to excessive mycelial and sporodochial growth and reached the macrospore stage, but would develop no farther; whereas ascospores and conidia grown on potato + 1 per cent. glycerine gave rather less mycelial growth and developed perithecia within two months.

Other media containing starch or derivatives of starch were tried with and without glycerine, such as acid pea-agar and acid haricot bean-agar (0.1 per cent. N. HCl), prune juice, oatmeal-agar, &c., with the same result.

The agar-glycerine media were the first to be tried, but these cultures had to be fixed and cut before the asci had developed, on account of the drying out of the medium, and the central cells of the perithecium, which will be more fully described later, showed all the characteristics of normal young perithecia on bark. After these initial trials the potato + 1 per cent. glycerine medium was used throughout the rest of the investigation, as it was found to give the most satisfactory results. Ascospores from freshly gathered dehiscing perithecia on bark in the spring of 1918 completed two life-cycles from ascospore to ascospore during the summer months on the potato-glycerine medium.

The potato slopes were prepared as follows: A wad of absorbent cotton-wool was placed at the bottom of the test-tube and the potato on the wad. A sufficient quantity of 1 per cent. glycerine solution in distilled water was poured into the tube so as to completely cover the whole of the potato, then the tubes were autoclaved and stored until required. Before inoculation the superfluous glycerine solution was poured off. In this way there remained sufficient moisture to last throughout the period of development, if taken out of the incubator after the first two or three days. What effect the glycerine has on the development of the fungus is not known.

After inoculation, the culture tubes were placed in the incubator and kept at a temperature of 25° C. for the first few days, and then left on a shelf in the laboratory, where they were exposed to direct sunlight until 11-12 o'clock in the day during the summer months, in order to give them, as far as sunlight was concerned, approximately the same conditions as the tree from which the fungus had been obtained. This treatment proved highly satisfactory. It was then thought that perhaps a little mechanical pressure might hasten the development of perithecia, as, in the host plant, the mycelium of the fungus is very densely coiled and develops in considerable quantity before the bark is ruptured. Some of the cultures were pressed against the sides of the test-tubes with a sterile glass rod. The fungus, however, developed so well without the pressure, and the different cultures varied as to the amount of mycelial development, probably owing to the small differences in moisture content, size, and other properties of the potato slopes, that it was not possible to come to any definite conclusion on this point without further work under much more stringent standard conditions. During the process of elaborating a suitable medium, the possibility of a symbiotic relationship between a bacterium and *N. galligena* had to be considered, more especially as the microtome preparations of *Nectria* growing on bark frequently showed the presence of a rod-shaped

bacterium in the sporodochium of the fungus. Brzezinski (8) published a paper in 1903 in which he stated that apple canker was a bacterium and not a *Nectria*. This was contradicted by Aderhold (2). The present writer also found that typical sunken cankered areas could be induced in young one-year-old twigs of apple in the open, when inoculated in spring with macrospores obtained from a pure culture. Other inoculations with bacteria isolated from platings of macrospores from bark were tried at the same time, but the wounds healed up naturally and no ill effects could be seen. The inoculated portions of the twigs were enclosed, in both cases, in sterile glass cylinders plugged at each end with cotton-wool to prevent risk of infection from other sources. Various bacterial growths from platings of macrospores were also tried with pure cultures of *N. galligena*. The cocci were discarded and only rod-shaped bacilli used. Tubes infected with spores of the fungus and bacilli at one and the same time showed that the bacilli developed rapidly at the expense of the fungus. Further work on these lines was, however, discontinued when it was found that *N. galligena* would develop perithecia in pure culture on media containing glycerine.

LIFE-HISTORY.

The genus *Nectria* is placed by Saccardo in the family Hypocreaceae, a sub-group of the Pyrenomycetes.

It has a complicated life-history which may be divided roughly into three distinct stages, which occur in the following sequence :

1. The microspore stage, with minute elongate oval hyaline spores, $5\text{--}7\ \mu \times 1\text{--}2\ \mu$, abstricted from fine hyaline mycelium, sparsely septate and inclined to monopodial branching (Fig. 7).

2. The macrospore stage, with 6–7 septate curved spores, $6\text{--}7\text{--}7\text{--}5\ \mu \times 4\text{--}5\ \mu$, abstricted from branched conidiophores on a sorus or sporodochium consisting of coarse, densely intertwined, thick-walled mycelium, brownish red in colour when mature (Figs. 1, 4, 10a, 11, 12, 12a, 13, 13a, 13b, 24).

3. The perithecial stage, which occurs on the same sporodochium as the macrospores. The red, flask-shaped perithecia contain paraphyses and numerous eight-spored asci. The ostium is raised, rather darker in colour than the rest of the perithecium, and is furnished with periphyses. The ascospores emerge through the ostium in the form of whitish buff tendrils. The ascospores, $15\text{--}21\ \mu \times 6\text{--}8\text{--}5\ \mu$, are two-celled, with roughened walls slightly tinged with yellow (Figs. 2, 3, 5, 9, 14, 15, 16, 18–22).

All these stages have been described by previous workers, but during the present investigation two-celled multinucleate spores have also been observed both in pure culture and in preparations on bark (Fig. 8). Unstained, these could not be distinguished from two-celled macrospores, hence their function, if any, could not be traced.

DEVELOPMENT ON ARTIFICIAL MEDIA.

Microspore Stage.

All stages in the life-history of *N. galligena*, as described by Hartig and Goethe under the name of *N. dilissima*, were observed to occur in pure culture. Ascospores, obtained from tendrils of dehiscing perithecia on apple bark, sown on potato + 1 per cent. glycerine, give rise in the course of a few days to a web of fine, spreading, hyaline mycelium. This mycelium is sparsely septate, and the branching more or less monopodial. Unicellular microspores, $5-7 \mu \times 1-1.5 \mu$, are abstricted from the tips of the fine hyphal branches, and not from differentiated conidiophores. The function of these microspores is not known.

As the fungus colony grows, coarser thicker-walled mycelium develops which is much more septate than the above. The cell-walls are tinged with yellow and the cells contain numerous oil-drops. Branched conidiophores (Fig. 11) arising from a sporodochium of closely interwoven hyphae give off enormous numbers of septate macroconidia, the *Fusarium Willkommii* stage of Weese (34). In the host plant this stage occurs during the months of September and October, or even later if the weather is open (Fig. 1). The macroconidia when fully developed are hyaline, slightly curved with rounded ends, 5-7 septate, $65-75 \mu \times 5-5.5 \mu$ (Fig. 10), and each cell is uninucleate at first. These macrospores can be easily distinguished from the microspores. They are considerably larger, although all stages from the unicellular to the multicellular macrospores have been observed both on artificial media and on apple bark. The first-formed macrospores on a young sporodochium as a rule have fewer septae.

Macrospores grown in a hanging drop germinate mostly from the terminal cells at either end of the spore (Fig. 13 *b*). The middle cells, under moist conditions, do not germinate very readily. Cultures made from macrospores give rise, in their turn, to microspores and follow the same sequence of stages as cultures made from ascospores. This is the case when macrospores drop away from the sporodochium. But under certain conditions, when undisturbed, as is mostly the case on artificial media, and also in the open in calm autumnal weather, a considerable number of macrospores remain *in situ* and anastomose by throwing out connexions from their central and even their terminal cells (Fig. 13). Only one cell may link up with another cell of a neighbouring spore, or several cells of one spore may be linked with cells of a neighbouring spore or spores. The result is the formation of a palisade pseudo-tissue formed by linked macrospores. This linking is of very common occurrence both on bark and artificial media. This palisade tissue helps to increase the bulk of the sporodochium. At this stage the behaviour of the nuclei of the macrospores is interesting but little understood. The passage of the nucleus from the

cell of one spore to that of another has never been observed, but frequently one cell is seen to contain two nuclei, and the cell to which it is linked is enucleate (Fig. 10 *b*). Also, one or all the cells of a macrospore, when mature, have often been seen to contain two nuclei, but this was thought to be due to the division of the primary nucleus (Figs. 10 *a* and 12 *a*). Macrospores which are quite free and unlinked can have binucleate cells (Figs. 10 *a* and 12 *a*). It is not easy to suggest what this peculiar behaviour indicates. At first it was thought that in *Nectria*, since the perithecia arise on the same sporodochium as the summer macrospores, this fusion of macrospores might possibly be a sexual fusion from which the perithecia eventually arise. There is, however, no proof of this supposition. The fungus is so small, and the mycelium of the sporodochium so twisted and dense, that in spite of prolonged search it was found impossible to trace the origin of the mycelium or cells from which the perithecium arises, or to find any definite connexion except one of position.

Variation in size and shape of the mature macrospores has been described and figured by previous workers in *Nectria* and other forms of *Fusarium* (Fig. 13 *a*). One or more cells in the middle of the spore can become enlarged, with thickened walls and deeply staining rich cell contents; also bodies were observed by the author among the macrospores on a well-developed sporodochium, consisting of a stalk cell and three to four enlarged circular cells, the terminal or penultimate cell of which contained rich cell contents. These bodies certainly did not give the impression of being degeneration forms, but it is impossible to say whether they perform any special function. Lapine thought the enlarged central cells of the macrospore to be of the nature of chlamydospores, but Aderhold, on the other hand, considered them to represent degeneration stages.

Perithecial Stage.

The perithecium arises from a tangle of coiled hyphae (Fig. 23), which appear to branch off from the thick-walled sporodochial mycelium. No differentiated archicarp has ever been observed to which the perithecium could be traced. The sporodochium forms a thick and complex layer of twisted hyphae with walls of varying thickness, and it is not possible to tell which particular knot of hyphae will eventually develop into a fertile perithecium. Sterile perithecia-like bodies occur both on bark and artificial media; they consist of the usual outer layers of brown, thick-walled, deeply staining mycelium, enclosing the thinner-walled hyphae with uninucleate cells. The nuclei of these central cells soon disappear and no further development occurs except increase in size. Schaffnit (27) observed similar bodies in *Fusarium nivale* and considered them to be sclerotial organs. Not infrequently, however, one or more small fertile perithecia have been

observed developing in the internal tissues of the sterile perithecium, more generally near the upper part of the periphery. The rest of the sterile perithecium serves to increase the bulk of the sporodochium; thus sections of sporodochia in pure culture often show layers of perithecia with walls of varying thickness. The fertile perithecium originates in the same way, and consists of the thick-walled outer layers and the thin-walled internal tissues with uninucleate cells. The nuclei of these cells are distinct and homogeneous and stain readily with the usual nuclear stains. Some of these cells eventually become absorbed, while others appear to divide and form several layers of delicate tissue immediately beneath the perithecial wall. On potato-glycerine the growth of the sporodochium is much looser, hence the knots of hyphae which give rise to perithecia can be more easily distinguished. The appearance of the fungus, however, in no way tends to show that the conditions are not conducive to normal development. In the earliest stages, one or more cells in these knots are larger and sometimes binucleate (Fig. 23), but in such rapidly growing tissue this binucleate condition is probably due to division of the primary nucleus prior to cell division. The development of the first stages of the perithecium is rapid, and difficult to follow. Before any disintegration of the central cells of the perithecium occurs, the ascogonia begin to develop (Figs. 15, 16, 19-22), and are easily distinguishable from the surrounding tissues, owing to their larger cells and denser cell contents. They are coiled multicellular structures with multinucleate cells, and the nuclei show a definite nucleolus and nuclear area. There is more than one ascogonium in the perithecium. In two instances three ascogonia could be counted with a fair degree of certainty, but the perithecia are so small at this stage, and the ascogonia so much intertwined, that it is difficult to trace each ascogonium throughout its length through a series of sections, and to determine whether the number of ascogonia varies in different perithecia.

The ascogonia originate from thin-walled tissue at the base of the perithecium, and appear to push their way up amongst the central cells (Fig. 15). Some of the latter begin to disintegrate. Fig. 15 shows the bases of two young ascogonia in a young perithecium, and disintegrating central cells. It was not possible to determine whether the ascogonium begins as one cell and becomes multicellular by growth and cell division, or whether it is a multicellular structure from the first, being gradually differentiated from a row of central cells. No case of an incipient unicellular ascogonium was observed, but the examination of a considerable number of young perithecia in different stages led to the conclusion that the multicellular condition was most probably due to growth and cell division of a differentiated primary cell. The cells of the ascogonia increase in number as the perithecium develops, until a stage is reached in which the interior of the perithecium is filled with a considerable number

of large multinucleate cells (Fig. 5). Portions of the ascogonia appear to be narrower and stain more deeply than others, but whether these portions are of the nature of trichogynes, or whether they are the portions which are growing the most actively, could not be determined. No nuclear fusions were observed in the ascogonia, but the nuclei tend to associate in pairs as the ascogonial cells mature (Fig. 5). In one instance only (Fig. 22) there were indications of the passage of the nucleus of one cell to another. As will be seen in the figure, there is a large and very definite nucleus in one cell and a definite pore in the cell-wall, through which a nucleus of a neighbouring cell is about to pass. It will also be seen that some ascogonial cells are in a more advanced stage of disintegration than others. The culture from which this preparation was made was grown on potato-glycerine from ascospores obtained from dehiscing perithecia on bark, and was thirteen weeks old. Figs. 19, 20, 21, 22, are consecutive sections of the same perithecium cut $3\ \mu$ thick.

The ascogonia do not appear to function, in that they do not give rise directly to ascogenous hyphae. Two or more ascogonial nuclei pass into the hyphae which grow out from the ascogonial cells. The ascogonia gradually degenerate and the hyphae in their turn completely fill the cavity of the perithecium. The actual ascogenous hyphae which give rise to asci arise *de novo* at the base of the perithecium, from cells which contain two or more nuclei showing the same characteristics as the nuclei of the ascogonia, namely, they have a well-marked nucleolus and nuclear area, and occasionally show chromatin granules. The origin of these basal cells could not be traced. The nuclei of these cells are of two sizes, large and small, as seen in Fig. 18, but it was not possible to determine whether the larger were the result of the fusion of two smaller nuclei. The smaller nuclei tend to associate in pairs at this stage also. The perithecium is completely filled with paraphyses before the asci begin to develop. In spite of careful search, no crozier formation or further nuclear fusions could be found prior to the development of the ascus, and the study of the nuclear divisions in the ascus had to be abandoned owing to difficulties in technique. All fixatives so far used for the purpose of fixing perithecia at the stage at which the asci develop either failed to penetrate the hard outer perithecial wall, or, if the penetration was satisfactory, microtome sections could not be cut sufficiently thin. The large number of paraphyses present and the extreme smallness of the whole structure made accurate observations impossible.

The ovoid perithecia contain numerous asci, which can be seen in all stages of development in the perithecium. The asci contain eight two-celled ascospores with thick, slightly roughened walls (Figs. 9, 14). The ascospores emerge as yellowish-white tendrils through the raised ostiolum at the apex of the perithecium. On the host plant perithecia begin to form in the late

autumn, develop slowly throughout the winter months, and dehisce in spring. Material gathered in February showed perithecia in all stages of development; in April the perithecia are mostly fully grown and about to dehisce.

PYCNIDIA.

With regard to the vexed question as to the occurrence of pycnidia in the life-history of *N. galligena*, pycnidia have been seen from time to time in preparations of the fungus growing on bark. They occur as a rule in close proximity to the perithecia, and appear to originate from the same sporodochium (Fig. 16). So far, no mature pycnidia containing spores have been observed in any of the pure cultures on artificial media. On bark, the pycnidial wall consists of one or two fairly regular layers of thick-walled cells (Fig. 17), whereas the young perithecial wall contains more than two outer layers, is much more irregular, and the transition from the outer layers to the thin-walled central cells is more gradual than is the case in the young pycnidium. Also the internal tissues of the young perithecium vary considerably in size of cell and capacity for taking up stains, as compared with the central portions of the pycnidium, which stain much more uniformly. It is, however, somewhat difficult to distinguish between pycnidia and immature perithecia, more especially when the latter are filled with paraphyses and the sections happen to be transverse or oblique. On artificial media, owing to the more irregular growth of the tissues of the sporodochium, and the variability in thickness of the walls of young perithecia, immature pycnidia may possibly have been overlooked. One or two doubtful cases occurred, but it was impossible to arrive at any definite conclusion. If pycnidia do occur in the life-history of *Nectria galligena*, of which there is not sufficient proof, they are probably abortive, as the fungus will complete its life-cycle from ascospore to ascospore on artificial media without the development of pycnidia. The unicellular pycnosporos (Fig. 17) are abstricted from simple unbranched sporophores in acropetal succession.

TWO-CELLED MULTINUCLEATE SPORES.

At the stage in which the young perithecia begin to develop, two-celled multinucleate spores (Fig. 8) have been observed both on bark and artificial media. Eight or more nuclei occur in each cell, when the spore is mature. Unstained, they cannot be distinguished from two-celled macrospores, and no signs of germination could be seen in stained preparations.

SUMMARY.

The fungus described in this paper was isolated from canker on apple, and agrees in morphological and biological characteristics with *N. galligena*,

Bresadolæ, described by Wcese as causing apple canker, with the exception of the somewhat larger dimensions of the ascospores.

The fungus will complete its life-history on media containing starch or a derivative of starch with 1 per cent. glycerine.

No differentiated archicarp was observed to which the development of the perithecium could be traced; the perithecium arises from a coil of vegetative hyphæ in the sporodochium.

Several ascogonia occur in the young perithecium; these degenerate and disappear before the formation of the asci.

The ascogenous hyphæ, from which the asci develop, arise *de novo* from cells at the base of the perithecium, the nuclei of which have the same characteristics as the nuclei of the ascogonia.

The further development of the perithecium could not be followed.

Besides the three different kinds of spores known to previous investigators, a fourth form, a two-celled multinucleate spore, was observed.

Pycnidia occur on bark, but no mature pycnidium was seen in preparations of the fungus in pure culture on artificial media.

Except in section, pycnidia on bark cannot be distinguished from young perithecia, and although in some instances they appear to develop on one and the same sporodochium, there is no conclusive evidence that pycnidia occur in the life-history of *N. galligena*.

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EXPLANATION OF PLATES IV AND V.

Illustrating Miss Dorothy M. Cayley's paper on the Life-history of *Nectria galligena*, Bres.

The preparations are from material fixed in Flemming's fluid, and stained with iron-alum-haematoxylin, unless otherwise stated.

PLATE V.

- Fig. 1. Summer or macrospore stage on bark. $\times 2\frac{1}{2}$.
- Fig. 2. Winter or perithecial stage on bark. Perithecia beginning to dehisce. $\times 4\frac{1}{2}$.
- Fig. 3. Sporodochium and cluster of young perithecia on potato-glycerine. Fixed and stained.
- Fig. 4. Sterilized twig of Cox's Orange apple infected with pure culture of *N. galligena*. Fixed and stained. (a) Mycelium pushing up the cuticle. (b) Twisted mycelium penetrating the tissues of the cortex. (c) Cuticle. (d) Inter-cellular mycelium. (e) Intracellular mycelium. (m) Young macrospore.
- Fig. 5. Young perithecium on bark. Central region filled with ascogonial cells. Note nuclei associating in pairs. Fixed and stained.
- Fig. 6. Pure cultures of *N. galligena*. (a) Young culture from ascospores on oatmeal-agar + 1 per cent. glycerine, 7 days old. (b) Older culture from ascospores on oatmeal-agar + 1 per cent. glycerine. Perithecia beginning to develop. 31 days old. (c) Cultures from ascospores on potato-glycerine medium. 31 days old.

PLATE VI.

- Fig. 7. Microconidia and monopodial sporophore. $\times 1000$. Fresh material.
 Fig. 8. Multinucleate bicellular spore. $\times 1,000$. Fixed and stained.
 Fig. 9. Ascospores. $\times 1,000$. Fresh material.
 Fig. 10. (a) Macrospores. $\times 1,000$. Stained *intra vitam*. (b) Large macrospores, showing one cell with two nuclei, with a neighbouring enucleate celled. Fixed and stained.
 Fig. 11. Young macrospores on branched sporophores. $\times 1,000$. Fresh material.
 Fig. 12. Macrospore giving rise directly to another sporophore and macrospores. $\times 700$. Fresh material. (a) Mature macrospore with three binucleate cells. Stained *intra vitam*.
 Fig. 13. (a) Linked mature ascospores, with cells containing oil drops, forming pseudo-palisade tissue, destined to become sporodochial tissue. $\times 800$. Fresh material grown on oatmeal-agar. (b) Various metamorphosed macrospores. Stained *intra vitam*.
 Fig. 14. Asci in various stages of development. Fixed and stained.
 Fig. 15. Young perithecium, showing bases of two very young ascogonia with multinucleate cells. Grown on potato-glycerine medium. $\times 1,300$. Fixed and stained. Drawn camera lucida.
 Fig. 16. Two perithecia, one on either side of a pycnidium, on bark. (a) Young perithecium with ascogonia, (b) Older perithecium filled with paraphyses before the development of asci. (c) Pycnidium. Drawn camera lucida. $\times 310$.
 Fig. 17. Portion of the pycnidium in Fig. 16, showing pycno-sporophores abstricting pycno-spores in acropetal succession. $\times 1,500$.
 Fig. 18. Cells of the hypothecial layer at the base of the perithecium containing large and small nuclei, from which the asci develop. $\times 1,800$. Fixed and stained.
 Figs. 19, 20, 21, and 22. Consecutive sections of a young perithecium, cut $3\ \mu$ thick, showing multicellular multinucleate ascogonia. One ascogonium is degenerating. Grown on potato-glycerine medium. $\times 1,600$. Fixed and stained; drawn camera lucida.
 Fig. 23. Early stage of perithecium, coiled hyphae. Fixed and stained. $\times 1,000$.
 Fig. 24. Linked macrospores, showing one cell with two nuclei. Fresh material stained *intra vitam* with sat. erythrosin in glycerine.



1.



2.



3.



a

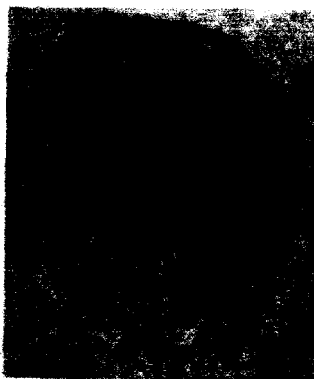
b

c

6.



5.



Each cell.

Studies in the Energy Relations of Plants.

I. The Increase in Area of Leaves and Leaf Surface of *Cucumis sativus*.

BY

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With fifteen Figures in the Text.

A. Introduction.

IN view of the important rôle which the leaf surface plays in the economy of the plant it is surprising that no quantitative studies have yet appeared dealing with the increase in area of leaves of a plant measured from day to day. The difficulty which confronts the experimenter is to devise a method for obtaining the area of leaves without detaching them from the plant or injuring them in any way. The method here described was gradually elaborated in the course of a research on the physiology of forced plants carried out at the suggestion of Prof. V. H. Blackman at the Experimental and Research Station, Cheshunt, Herts., where the data for plants grown in sunlight were collected. The plant investigated was a variety of cucumber (*Cucumis sativus*, var. Butcher's Disease Resister).

B. Method of estimating Leaf Areas.

The shape of the leaf is regarded as an approximation to a definite geometrical figure, of which the dimensions can be obtained and so the area calculated by the use of a suitable formula.

Cotyledons. These approximate closely to ellipses, and the lengths of the major and minor axes (Fig. 1, 2 a, 2 b) are obtained directly by applying a 'straight-edge' marked in centimetres and millimetres. The major axis was taken as the length of the cotyledon from apex to base. The junction of the median and lateral veins was selected as the point at the basal end of the cotyledon from which to measure. The minor axis was taken as the greatest width of the cotyledon. It has been the rule in this work to measure only to the nearest millimetre, as the errors of the method, which are discussed below, do not require any closer approach to accuracy. The



FIG. 1.

area of the cotyledons is derived from the formula $A = \pi ab$, where a, b are half the major and minor axes respectively.

Foliage leaves. The shape of the foliage leaf is, in its early stages, approximately an irregular hexagon, but later the basal lobes develop, and two new points appear at F and G (Fig. 2), so that the leaf becomes more or less octagonal in shape. As a hexagon it is necessary to obtain three linear and two angular measurements in order completely to define the figure.

The linear measurements, again taken with a ruler to the nearest millimetre, are:

- (1) the length of the leaf from apex to basal junction of veins (AO, Fig. 2);
- (2) the width between the anterior lateral points (BD);
- (3) the width between the posterior lateral points (CE).

The necessary angular measurements are:

- (4) the angles subtended at base by lines joining the anterior and posterior lateral points, i.e. angles BOD, COE; half these angles constitute the angles α and β .

The angular measurements were taken with a celluloid protractor, graduated to 10° by lines to the centre, and at the margin in single degrees. With such an instrument it is possible to measure to the nearest 10° directly, and by approximation to the nearest degree. The centre of the protractor is placed at the basal junction of the veins, and the protractor held so that the base line passes through one of the points of the leaf, say B, and the angle to be measured, in this case BOD, can be read off direct. After some practice the measurements can be made very rapidly, and without injuring the leaf in any way.

The following formula gives the area:

$$A = \frac{b}{2} \left\{ a + \frac{c}{2} (\cot \alpha - \cot \beta) \right\} \quad \dots \quad (1)$$

The basal points F, G are rapidly developing when the leaf has attained the length of about 10 cm., and it is necessary then to utilize another formula to secure sufficiently close approximation to the true area. The leaf is then octangular with re-entrant angle at O (Fig. 3), and to define this figure another linear and another angular dimension are required.

These new dimensions are:

- (5) the width across FG (d);
- (6) the re-entrant angle FOG (2γ).

The formula for the area then becomes:

$$A = \frac{b}{2} \left\{ a + \frac{c}{2} (\cot \alpha - \cot \beta) \right\} + \frac{cd}{4} (\cot \beta + \cot \gamma) \quad \dots \quad (2)$$

Occasional difficulties in manipulation are met with. A constant source of difficulty is the tendency for the points of young leaves to curl

inwards, and great care is needed in these cases to avoid injury in measuring. It sometimes happens that leaves are markedly asymmetrical, so that the median vein does not bisect the angles subtended at the base by the lateral points. In such cases the same procedure was followed as if the leaves were of normal shape.

Care must be taken not to confuse the angles subtended by the points with the angles between the lateral veins running to the points, since these are smaller than the angles required.

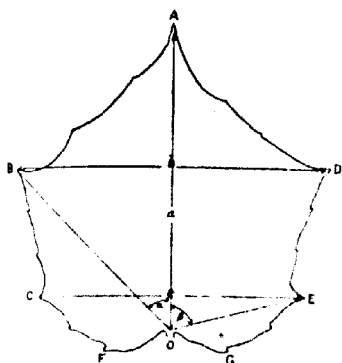


FIG. 2.

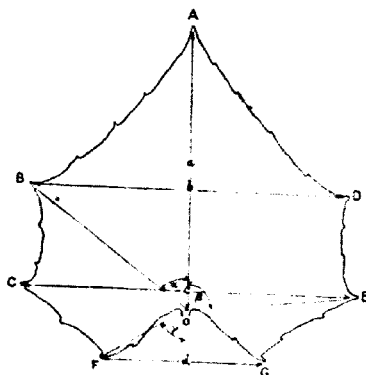


FIG. 3.

C. Experimental Procedure.

The results presented below are based on the results of a series of experiments carried out in one of the experimental greenhouses of the Experimental Station, Cheshunt.

Ten plants were kept under observation in each experiment. The method of cultivation was that usually adopted in horticultural practice. The seeds were not graded by weight, but were selected for plumpness and uniformity. During their entire course of development the plants were kept at the south end of the greenhouse and were fully exposed to sunlight. The plants were thus kept under perfectly normal conditions. The temperature of the house was recorded by a thermograph, and the radiation by a Callendar radiometer.

Three sets of experiments were started on 10/11/16, 21/2/17, and 11/6/17 respectively, and each experiment was carried on for 30 days. The dimensions of the leaves were taken each day at 10 a.m. A specimen entry from data of Expt. III for July 2 is shown on p. 96.

TABLE I.

Leaf number.	Number of Plant.										Average.
	1	2	3	4	5	6	7	8	9	10	
2	15.0 15.4 17.9 12.2	13.7 14.5 15.3 10.8	14.4 15.0 16.0 11.4	13.8 14.8 15.9 11.4	14.8 15.8 16.6 11.7	13.7 13.3 15.7 10.6	13.5 13.9 15.4 10.6	13.8 14.3 15.5 9.9	13.7 14.8 16.9 12.2	14.0 14.4 15.8 10.6	14.0 14.6 16.1 11.1
3	17.6 20.5 19.5 12.1	16.8 16.9 18.4 12.0	15.4 17.4 16.9 10.4	16.8 18.5 18.4 12.2	17.1 17.7 19.0 12.4	16.2 17.4 18.2 11.0	15.9 17.4 16.9 10.5	14.6 16.3 15.5 9.4	16.6 18.5 19.3 12.4	14.3 16.4 16.3 10.0	16.1 17.7 17.8 11.2
4	17.3 19.4 19.2 12.3	16.6 18.6 17.1 11.1	15.0 17.6 15.5 9.6	15.2 18.0 16.5 11.4	16.6 17.8 18.4 12.7	14.3 16.8 15.7 10.5	14.2 16.4 16.8 11.5	14.3 16.4 15.8 11.0	16.2 18.6 18.6 12.2	13.9 17.0 14.9 9.4	15.4 17.7 16.9 11.2
5	15.9 18.6 17.2 12.1	15.2 16.9 16.6 11.3	15.3 16.7 15.5 10.5	15.3 18.4 17.4 12.2	16.9 18.0 17.7 13.1	14.0 15.8 15.2 10.4	13.6 16.2 17.0 12.6	14.5 16.0 15.7 10.6	15.6 16.8 14.0 11.5	13.9 15.8 15.0 10.4	15.0 16.9 16.1 11.5
6	10.0 12.3 9.3 —	14.0 15.9 15.2 10.3	13.4 16.1 15.2 9.4	13.0 14.8 14.2 9.2	11.2 12.8 11.1 6.5	10.9 12.8 11.5 6.6	12.1 13.8 12.8 8.2	12.5 14.0 13.8 8.7	12.6 14.2 13.3 9.0	12.2 14.4 13.2 9.2	12.2 14.1 13.0 8.6
7	6.5 7.5 6.4	10.7 13.0 11.8	10.2 12.8 12.4	10.0 11.5 9.5	7.5 8.5 7.6	7.2 8.6 6.9	8.8 10.7 7.9	9.5 10.7 9.3	9.5 10.5 8.9	9.4 10.9 10.0	8.9 10.5 9.1
8	4.6 4.2 3.5	7.8 9.3 7.9	8.5 9.8 8.3	6.1 6.8 5.5	5.5 5.0 4.1	4.8 5.2 4.0	5.7 6.2 5.0	6.4 7.2 5.9	6.2 6.7 5.8	6.8 7.9 6.0	6.2 6.8 5.5
9	3.5 3.4 2.5	5.2 5.4 5.3	5.6 5.5 4.7	4.4 4.0 3.5	3.9 3.2 2.5	3.4 3.1 2.5	4.0 3.8 3.2	4.5 4.2 3.8	4.4 4.3 3.6	4.2 4.1 3.3	4.5 4.1 3.5
10	3.7 2.3 1.6	3.5 2.3 2.5	3.9 3.7 3.0	3.5 3.0 2.2	3.0 2.8 1.8	2.5 2.1 1.3	3.0 2.2 1.8	2.8 2.8 1.6	3.3 3.5 3.4	3.4 2.8 2.2	3.3 2.5 2.1
11	— — —	— — —	3.2 2.6 1.7	2.4 1.9 1.3	— — —	— — —	— — —	— — —	2.2 1.8 1.5	— — —	2.6 2.1 1.5

The columns 1-10 contain the entries for the linear dimensions of the leaves of the ten plants on the day specified, and, reading down each column, the three or four linear measurements of successive leaves from the oldest onwards are found. The entries for the cotyledons and first leaf have been omitted, as by this date they had attained their maximum size. In the last column, marked 'average', are the means of the numbers occupying similar positions in each of the columns 1-10; for example, the first entry, 14.0, is the mean of the numbers 15.0, 13.7, 14.4, &c., and represents the mean value of 'a' for the second leaf.

From these mean values of the dimensions for each leaf the mean value for the area of each leaf is calculated. By adding together the mean

areas for successive leaves the mean total leaf surface for each day of the experiment is obtained. It is obviously less laborious to calculate the mean areas of the leaves from the mean dimensions than to take the average of the areas of the single leaves of each plant; also the value so obtained is more trustworthy, as it is less liable to error from irregularities in growth and from errors in measurement. In these experiments the angles were not measured each day, but assumed to remain constant during the course of development of the leaf, and the values obtained from the leaves on the last day of the experiment were utilized. The magnitude of the error introduced in this way will be considered below.

At the end of each experiment the leaves were removed from the plants, and their areas ascertained in the following manner. Tracings of the leaves were made on thin ground glass sheets immediately after removal from the plants. The pressure of the glass was adjusted so that the leaves were flattened out but not crushed, and this was done by raising the glass slightly at the edges. The only precaution necessary is to avoid parallax during tracing. In the case of certain leaves it is necessary to cut the leaf into portions in order to ensure its flatness.

By suitably illuminating the plate from below, the traces can be redrawn on tracing-paper, and the areas can then be measured with a planimeter. In this way the leaf area of each plant can be obtained on the last day of the experiment, and, by comparing the average of these with the mean area calculated from the mean dimensions of the leaves on the last day an estimate of the error of calculation is found.

D. *The Errors of the Method.*

From the nature of the method employed, the calculated values of the leaf areas will differ more or less from the real area.

Examining Figs. 2 and 3 once more, it is clear that the area calculated is that of the rectilinear figure A B C O E D, and this will equal the area of the leaf only when the areas of the irregular portions of the polygon external to the leaf happen to be equal to the irregular portions of the leaf excluded. The calculated areas are thus liable to chance errors, and it becomes important to estimate the effect of these errors on the trustworthiness of the results. From *a priori* considerations it is obvious that the negative discrepancies given by formula 1 will increase in magnitude as the areas of the basal lobes of the leaves become proportionately greater. On the other hand, formula 2 may give results which are too high for small leaves when the bights between the points of the leaf are great.

In order to investigate this point the areas of the leaves of the plants in Expt. III were compared at the end of the experiment with the actual area obtained with the planimeter. In this case, of course, the area of each

leaf was calculated direct from its dimensions and then compared with the area of its trace computed with the planimeter. In this way a series of nearly one hundred figures for leaf area was obtained by planimeter measurement, and by calculation from the two formulae. Table II shows the calculated and actual areas for the leaves of a single plant.

TABLE II.

Leaf No.	Area by calculation.		Area by Planimeter.	Error.	
	1st formula.	2nd formula.		1st formula.	2nd formula.
1 (9)	78.0	89.9	86.4	-9.8 %	+4.1 %
2 (9)	163.8	192.4	181.9	-11.3 %	+4.0 %
3 (9)	235.6	266.2	259.2	-9.1 %	+2.7 %
4 (9)	236.4	261.6	252.6	-6.3 %	+3.8 %
5 (9)	208.9	226.8	214.1	-2.4 %	+6.0 %
6 (9)	185.7	208.8	195.9	-5.2 %	+6.6 %
7 (9)	144.1	159.0	150.0	-3.9 %	+6.0 %
8 (9)	117.8	129.9	113.3	+4.0 %	+14.0 %
9 (9)	84.4	—	79.5	+6.2 %	—
10 (9)	56.2	—	53.0	+6.0 %	—
Mean errors				-3.3 %	+6.3 %
Error by first formula from regression equation =				-5.1 %	
" second " " " "					+4.8 %

These figures include areas of leaves in all stages of development. It will be seen at once that formula 1 gives both positive and negative errors, and that, as was to be expected, negative errors are associated with large, and positive with small, leaf areas. Formula 2, on the other hand, gives consistent positive errors related inversely in magnitude to the areas of the leaves.

To investigate the matter more thoroughly a statistical method was employed. A correlation table was drawn up with 'Area of Leaf' and 'Percentage Error' as the arrays and the correlation coefficient was calculated for estimations by either formula.

Formula 1 gives $r_1 = -0.74$,

" 2 " $r_2 = -0.120$.

The negative sign of the coefficient shows a tendency, as anticipated, for errors to decrease as the area increases. The high value of the coefficient for formula 1 indicates that there is marked negative correlation between percentage errors, and area as calculated from this formula; on the other hand, the correlation in the case of formula 2 is very low and barely significant.

The regressions of error on area in the two cases are as follows:

$$1. Y = -0.047X + 3.62;$$

$$2. Y = -0.007X + 6.27.$$

where Y is the percentage error, and X the area of the leaf. From the regression-equations were derived equations from which the error in square

centimetres could be calculated, and so a curve of errors was drawn from which the error of calculation for leaves of any size could be read off direct. For areas less than 80 sq. cm. formula 1 tends to give values which are too high, while for greater areas negative errors are introduced. Formula 2 tends always to give positive errors, which decrease relatively in magnitude as the area of the leaf increases.

Utilizing all the data available, and considering the percentage errors in the calculated areas as the differences, the probable error for a single determination by either formula, within the range of areas measured, was estimated. These errors were found to be as follows:

P.E. for single determination by formula 1 = ± 5.7 per cent.

" " " " " " " 2 = ± 4.7 per cent.

Each value for the leaf area is based on the average dimensions of ten corresponding leaves on separate plants, hence the value for the probable error of a single determination as stated above must be divided by $\sqrt{10}$, i.e. the probable error of each value for the total leaf area of a single plant is in the neighbourhood of ± 1.6 per cent. In the case of the June data the calculated area of the leaf surface per plant was 1,684 sq. cm.; the actual area measured with the planimeter was 1,659 sq. cm., giving a positive error of 1.5 per cent., which lies within the range of the probable error.

Errors introduced by failure to measure angles of leaf during the course of the experiment. From the leaf tracings taken at the end of Expt. III the angles α , β , γ were measured for each leaf of all the plants. The averages for angles of corresponding leaves are set out below in Table III.

TABLE III.

Leaf No.	2α .	2β .	2γ .
1	80.4°	132.6°	162.9°
2	80.5°	152.6°	146.4°
3	93.8°	162.5°	138.4°
4	95.0°	171.5°	137.7°
5	94.6°	162.1°	142.4°
6	98.1°	176.1°	135.1°
7	101.5°	179.0°	135.0°
8	101.4°	180.2°	133.4°
9	99.5°	180.9°	130.6°
10	101.7°	182.7°	—
Average excluding 1 and 2	98.2°	174.4°	136.1°

Leaves 1, 2, 3 were full grown at the end of the experiment, but the remainder were in various stages of development. Leaving the first two leaves out of account, there is among the remainder a fairly regular gradation in the angular magnitudes; α and β continually increasing, and γ decreasing. The maximum variations in 2α , 2β , and 2γ are 4°, 10.5°, and 11.2°.

and 5.5° respectively. The average magnitudes of the angles over the whole series are as follows:

$$\alpha = 49.1^\circ,$$

$$\beta = 87.2^\circ,$$

$$\gamma = 68.1^\circ.$$

Assuming dimensions $a = b = c = 100$, $d = 50$, and also the above angles, a hypothetical leaf area is calculated, and this is compared with the area of a leaf of same linear dimensions but with angles differing from the average dimensions by half the maximum variation noted above. In this way two areas are obtained differing by 2 per cent. in area. We may thus assume that errors of this order are introduced into the daily estimate of leaf area by utilizing end values for the dimensions of the angles, instead of measuring these each day. The foregoing considerations of the sources of error make it clear that the method is capable of yielding results significant to 5 per cent.

E. Growth of Leaves under Greenhouse Conditions.

In Fig. 4 is represented graphically the increase in time of the three dimensions, a , b , c , of an 'average' leaf, based on figures obtained by taking the geometric means of the daily average dimensions of the first five leaves; in Fig. 5, the increase in area in time of the third leaf.

The curves are of the S form which frequently occurs in graphical representations of growth phenomena. Robertson and Ostwald have shown that such curves can be represented by the equation of an autocatalytic reaction, and values calculated from an equation of this type are placed in Table IV, together with the geometric means of the dimensions of the first five 'average' leaves on successive days.

TABLE IV.

Days.	a (cm.).		b (cm.).		c (cm.).		Area (cm. ²).	
	Calculated.	Observed.	Calculated.	Observed.	Calculated.	Observed.	From dimensions.	From equation.
1	1.63	1.76	1.44	1.66	1.28	1.26	1.9	4.6
2	2.28	2.40	2.08	2.49	1.95	1.98	3.8	7.3
3	3.09	3.20	2.94	3.35	2.67	2.76	8.2	11.4
4	4.09	4.16	4.13	4.31	3.72	3.84	20.0	19.7
5	5.26	5.74	5.59	5.74	5.04	5.00	31.6	26.7
6	5.55	6.39	7.25	7.08	6.48	6.41	44.5	39.8
7	7.87	7.00	9.00	8.51	8.23	8.07	59.2	57.6
8	9.41	8.85	10.53	10.00	9.86	9.61	70.8	79.6
9	10.24	10.18	11.56	11.31	11.34	11.17	89.9	102.0
10	11.18	11.13	12.95	12.49	12.60	12.60	116.0	132.0
11	12.94	11.81	13.82	13.48	13.58	13.45	146.0	158.0
12	12.51	12.47	14.30	14.01	14.33	14.36	186.0	182.0
13	12.95	12.95	14.66	14.52	14.85	14.96	199.0	197.0
14	13.26	13.43	14.97	15.07	15.12	15.48	211.0	210.0
15	13.50	13.40	15.13	15.28	15.48	15.80	220.0	217.0
16	13.61	14.02	15.25	15.46	15.65	16.00	226.0	224.0

The observed and calculated values agree very well, showing that the

autocatalytic reaction formula will give a fair representation of the increase in linear dimensions of leaves.

In the curve of increase in area (Fig. 5) the points lie very satisfactorily

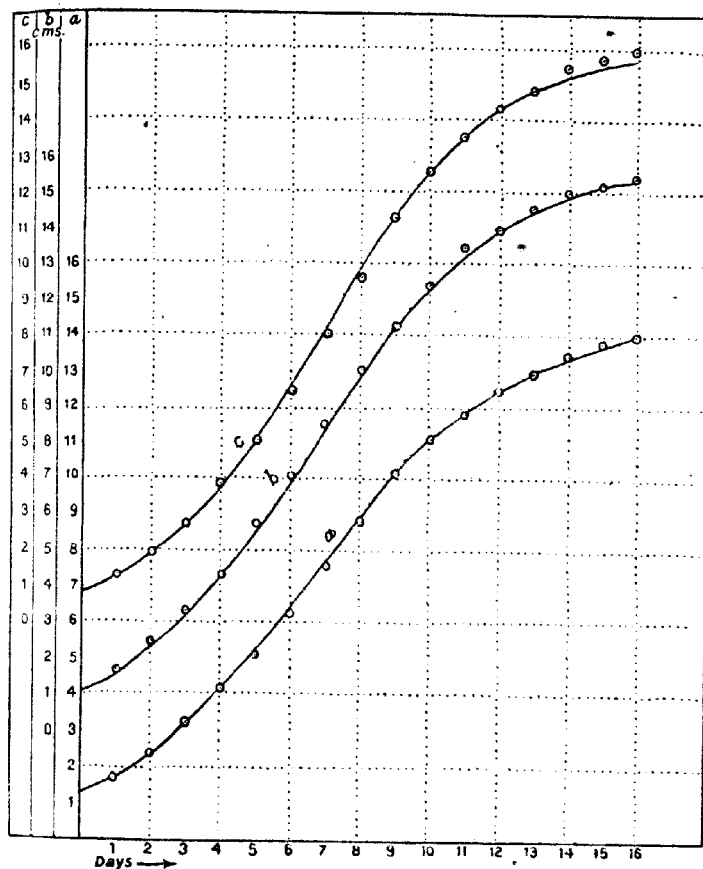


FIG. 4. Curves of increase in the linear dimensions of a leaf.

round the smooth curve, which represents the curve of closest fit of the autocatalytic type.

The phenomenon of the expansion of individual leaves is similar in all respects to the expansion of axial organs, and displays the same 'grand period'. This fact is clear from the S shape of the curves in Figs. 4 and 5, for if from these a curve were constructed showing the variation in the rate of expansion with increase in time, such a curve would have a maximum at

the time corresponding with the point of inflexion in the S curve, and would fall off on either side.

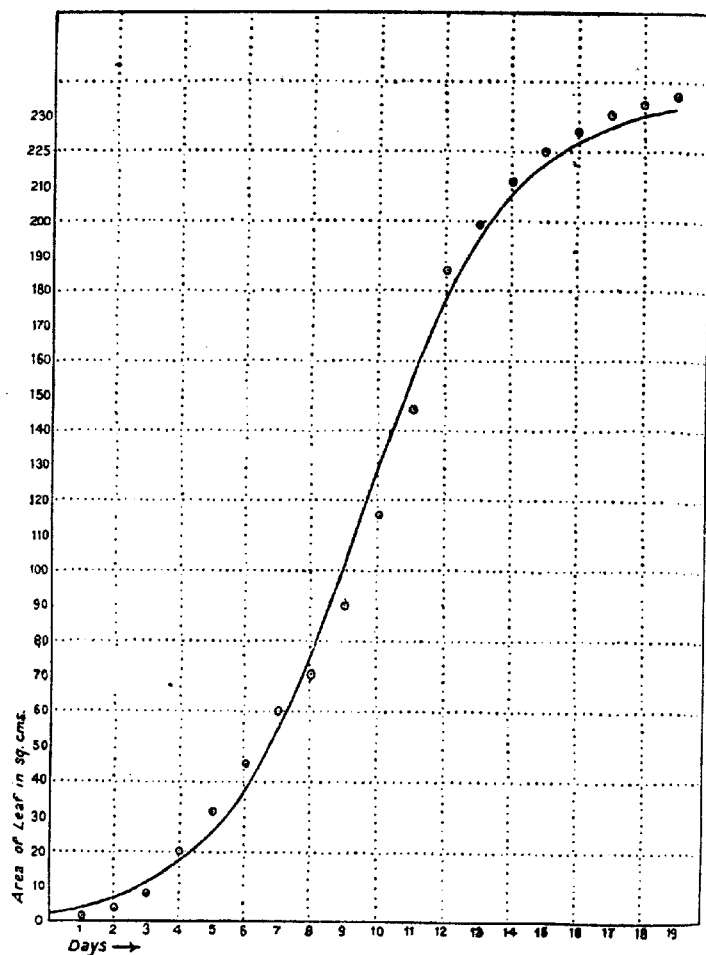
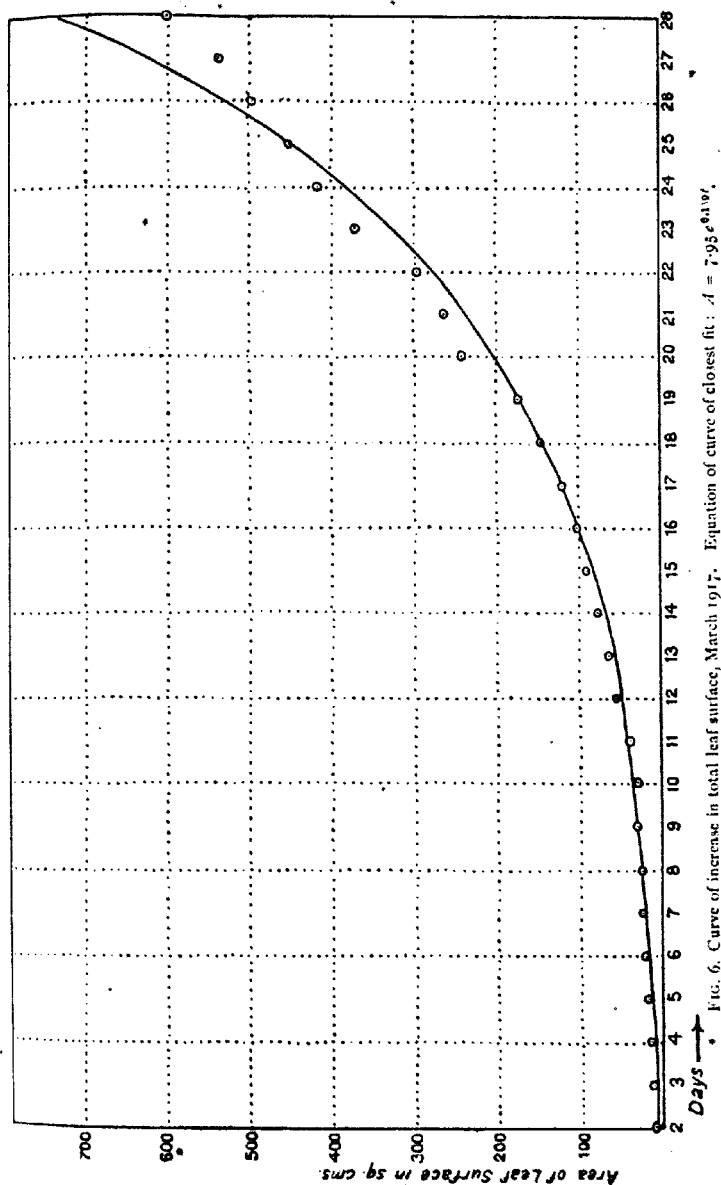


FIG. 5. Curve of increase in area of third leaf, June 1917. Equation of curve of closest fit:

$$\log_{10} \frac{236}{236-x} = 0.200(t-9.46).$$

F. *The Increase in Total Leaf Surface in the Greenhouse.*

The vital importance of the increase in leaf surface in the economy of the plant is evident from the following consideration: The total dry weight



of a plant, excluding the weight of reserve material in the seed, results almost entirely from the assimilatory activity of the leaf surface, and hence the development of the leaf surface must to a large extent determine the

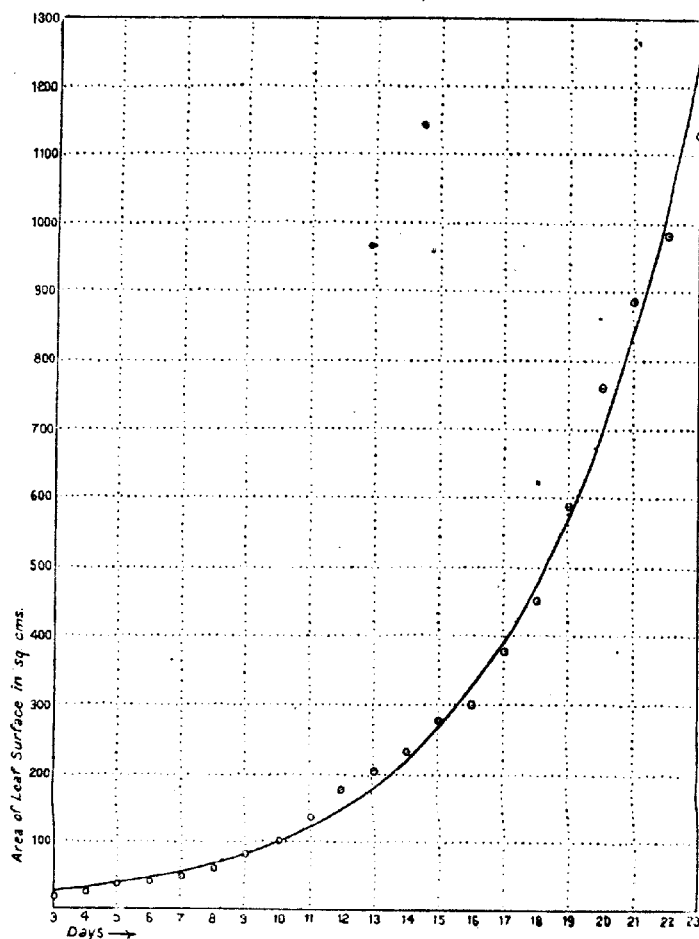


FIG. 7. Curve of increase in total leaf surface, June 1917. Equation of curve of closest fit:
 $A = 13.76e^{0.111t}$

rate of increase in dry weight of a plant. Considerable work on the rate of increase in dry weight, or 'Substanzquotienten', has been done by Kreuzler and his co-workers, Gressler, Hackenberg, Gericke, and Kiltz, but since, except in the case of Kreuzler's work, no data of the rate of increase

in leaf surface were collected, their work throws no light on the changes in the assimilatory activity of the leaf surface.

Figs. 6 and 7 show the curves of increase with time of the total leaf surface of the 'average' plant of the sets of experiments carried out in March and June respectively. These curves approximate closely to exponential curves of the type $A = ae^{rt}$, (1) where A is total area after time t , and a and r are constants, which may also be written in the form $A = ax^t$, (2) where $x = \log_e r$.

Hence we see as far as these experiments go the surface area of the plant increases according to a compound interest law. Further reference to this will be made later.

G. The Effect of External Conditions on the Expansion of the Leaf Surface.

It has been established by the experiments of Köppen on root growth in maize, and those of Miss Leitch on root growth in *Pisum sativum*, that the relation of growth to temperature closely approximates to an exponential one; hence, on the assumption that temperature is the controlling factor in leaf expansion, the logarithms of the 'average leaf areas'¹ should bear a linear relation to the average temperatures (geometric mean). This linear relation, however, does not hold, while, on the other hand, the proportionality between the total radiation falling on unit area during the experiments and the 'average leaf area' developed by the plants in the various experiments is very close. The following tentative suggestion was put forward as a result of the Cheshunt experiments in the Third Annual Report, Experimental and Research Station, Cheshunt, 1917: The 'average leaf area' attained by a cucumber plant is proportional to the total radiation falling on unit area during the period of growth.

The following table summarizes the results of the experiments performed at Cheshunt:

¹ By the term 'average leaf area' is meant the mean ordinate of the curve of increase with time of the total leaf surface (Figs. 6, 7, 10, 13), and the numerical value can be found approximately by summing the leaf areas for each day and dividing by the total number of daily observations. By 'total leaf area' of a single plant is meant the sum total of the areas of individual leaves of one plant on a particular day.

It is obvious that the 'average leaf area' is the area of the leaf surface of a hypothetical plant which will in a given time produce the same dry weight of material as a real plant, but without increasing in leaf area. This presupposes the same rate of carbon-assimilation per unit area for all the leaves of the plant.

TABLE V.

<i>Date of Experiment.</i>	<i>Leaf.</i>	<i>Average dry weight of plant.</i>		<i>Leaf area per plant at end of experiment.</i>	<i>Average leaf area per plant.</i>	<i>Intensity of Radiation.</i>	<i>Ratio.</i>	<i>Total radiation received during experiment.</i>	<i>Ratio.</i>	<i>Average temperature (geometric mean).</i>
		<i>Stem.</i>	<i>Total.</i>	<i>Leaf weight.</i>	<i>sq. dec.</i>	<i>per sq. cm.</i>		<i>per sq. cm.</i>		
10/11/16 to 9/12/16	—	—	0.345	—	1.83 sq. dec.	0.59 per min.	1	8.49 cal.	1	20.3° C.
21/2/17 to 29/3/17	1.806	1.133	2.939	0.63	7.44 sq. dec.	0.123 cal. per min.	3.4	2.470 cal.	2.9	21.8° C.
11/6/17 to 10/7/17	4.590	4.580	9.170	0.99	16.85 sq. dec.	0.226 cal. per min.	8.7	7.077 cal.	8.3	25.0° C.
1 lamp	—	—	0.0581	—	0.1624 sq. dec.	0.125 cal. per min.	1	4.682 cal.	1	35.0° C.
2 lamps	0.0682	0.0411	0.1093	0.60	0.2584 sq. dec.	0.216 cal. per min.	1.72	8.086 cal.	1.73	35.0° C.

H. Experiments under Artificial Light.

In order to test this hypothesis more closely experiments were undertaken under controlled conditions. These were carried out in the Greenhouse Laboratory of the Imperial College of Science and Technology. The temperature of the greenhouse is automatically controlled so that the variation in temperature need not exceed 2° F. Within this larger greenhouse is a smaller greenhouse in which the experiments were performed. The outside of this inner house was covered with dark cloth, so that the inner greenhouse was converted into a dark-room. The walls were hung with cloth screens over which water continually trickled, serving to keep the atmosphere very humid. A screen of black cloth divided the interior of the dark-room into two halves. In one was suspended a single half-watt 1,500 candle-power lamp, while two similar lamps were suspended in the other half. The air within the chamber was kept in continuous circulation by means of an electric fan.

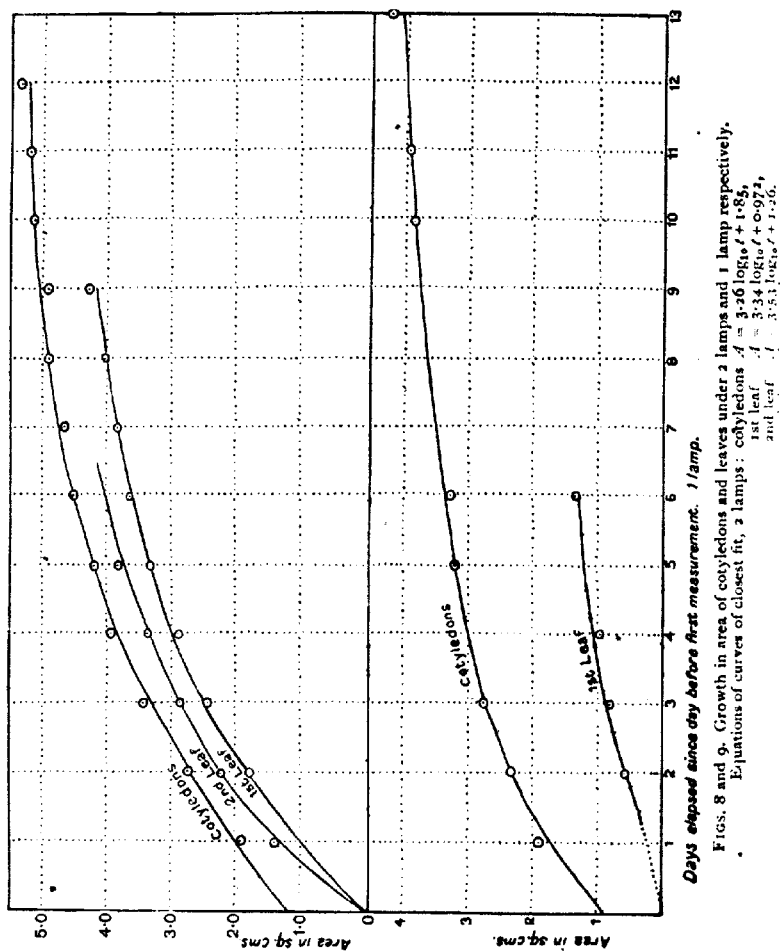
The same variety of cucumber was used as for the previous experiments. The seeds were graded; only those falling in the class 37-38 milligrams were utilized, and were sown singly in small earthenware pots, in a mixture of equal parts soil and well-rotted stable manure. 16 pots were allotted to each experiment, and these were placed in circles symmetrically about the single lamp and the pair of lamps respectively, the distance of the plants from the lamp filaments being 1 metre.

The seeds were sown on December 10, and germination was uniform, all the seedlings appearing on December 14. Leaf areas were determined according to the method described, but in these experiments the angular measurements as well as the linear were taken each day. Records of temperature and humidity were kept by self-recording thermographs and hygrometers, and the hygrometer readings were periodically checked by means of an Assmann psychrometer, good agreement being found. Except for inevitable slight fluctuations due to persons entering and leaving the dark-room, both temperature and humidity remained almost constant. The average temperature for the period was 95° F. (35° C.), and humidity 75 per cent. saturation.

Under such uniform conditions it is perhaps not surprising to find that the behaviour of all the plants was identical, all the first leaves appearing on December 17 and all the second leaves on December 20. This occurred quite synchronously in the case of all the plants, whether under the one or two lamps.

The plants were repotted into larger pots on December 27, and at this stage many plants were lost, as they did not recover after repotting. Only six plants out of each set survived, and, as they showed large variations in subsequent growth, the experiment was ended on January 4.

In Figs. 8 and 9 are shown the curves of increase in area of the cotyledons and the first leaves of plants grown under one and two lamps respectively. The points plotted are the calculated areas, and are based



on the average dimensions of corresponding leaves of all the available plants in the two cases. The smooth curves are the curves of closest fit calculated from all the daily observations. The discrepancies are seen to be small. In Tables VI and VII are found the areas of the cotyledons and foliage leaves on successive days for the average plant under two lamps and one lamp respectively.

TABLE VI.

Days.	Plants under 2 lamps.					
	Cotyledons.		First leaf.		Second leaf.	
	Calculated from dimensions.	From equation a.	Calculated from dimensions.	From equation b.	Calculated from dimensions.	From equation c.
	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.
1	1.88	1.98	1.24	0.97	1.34	1.26
2	2.73	2.67	1.75	1.78	2.20	2.32
3	3.42	3.30	2.42	2.56	2.85	2.94
4	3.93	3.80	2.86	2.98	3.36	3.38
5	4.14	4.20	3.28	3.31	3.82	3.73
6	4.48	4.50	3.63	3.57	—	—
7	4.65	4.73	3.82	3.79	—	—
8	4.87	4.90	4.05	3.99	—	—
9	4.90	5.02	4.28	4.16	—	—
10	5.14	5.12	—	—	—	—
11	5.21	5.18	—	—	—	—
12	5.34	5.23	—	—	—	—

$$\text{Equation a: } \log \frac{5.34 + x}{5.34 - x} = 0.149 (t + 1.2).$$

$$,, \quad b: \quad A = 0.972 + 3.34 \log_{10} t.$$

$$,, \quad c: \quad A = 1.26 + 3.53 \log_{10} t.$$

TABLE VII.

Days.	Plants under 1 lamp.			
	Cotyledons.		First leaf.	
	Calculated from dimensions.	Calculated from equation d.	Calculated from dimensions.	Calculated from equation e.
	sq. cm.	sq. cm.	sq. cm.	sq. cm.
1	1.88	1.73	—	—
2	2.36	2.37	0.55	0.52
3	2.74	2.73	0.79	0.81
4	—	—	0.95	1.01
5	3.14	3.22	—	1.16
6	3.26	3.38	1.33	1.29
10	3.84	3.86	—	—
11	3.93	3.95	—	—
13	4.27	4.10	—	—

$$\text{Equation d: } A = 1.73 + 2.13 \log_{10} t.$$

$$,, \quad e: \quad A = 0.937 + 1.61 \log_{10} t.$$

The curves of increase in area of individual leaves shown in Figs. 8 and 9 are strikingly dissimilar in appearance from the curve of increase in area for a leaf growing under natural illumination (Fig. 5). We are not dealing here with an S-shaped curve, and the autocatalytic reaction formula apparently will not meet this case. By suitable modification, however, a formula is obtained which empirically reproduces the course of the phenomenon in this case also.

The autocatalytic reaction is represented by the function

$$\log \frac{x}{A-x} = K(t-t_1), \quad (3)$$

where t_1 represents the time elapsed since beginning of the reaction until the maximum velocity is attained. It is from a function of this type that the values of leaf dimensions in Table IV were calculated. In this case A represents maximum length attained, x represents length at any time, t since the unfolding of the leaf. It is clearly not necessary to know the exact time of unfolding, since the same error is introduced into t as into t_1 , so that $(t-t_1)$ is correctly known. This function is derived by integration from the differential equation $\frac{dx}{dt} = Kx(a-x)$, which in our case represents the rate of growth. This rate will attain a maximum when $x(a-x)$ is a maximum, i. e. when $x = \frac{a}{2}$. The curve of the function (3) will therefore be S-shaped, symmetrical about the point of inflexion.

Enriques has shown that we may start from a differential equation of a more general form, namely,

$$\frac{dx}{dt} = a + bx + cx^2, \quad (4)$$

and by integration derive a more general function:

$$\log \frac{x+B}{A-x} = K(t-t_1), \quad (5)$$

which will represent an S curve no longer symmetrical, but with the point of inflexion corresponding with a value of $x = \frac{A-B}{2}$. As B approaches A in magnitude so the point of inflexion approaches the origin, so that

$$\log \frac{A+x}{A-x} = Kt \quad (6)$$

will represent a reaction with maximum initial velocity falling off with time. It is this modification which has been used to calculate the area of cotyledons in Table VI. The growth in area of individual leaves under artificial light thus falls into line with the growth in area under natural illumination. The increase in area of leaves under artificial light can, however, be represented by a much simpler function of type:

$$A = a + b \log_e t, \quad (7)$$

and it was from a function of this type that the curves of closest fit in Figs. 8 and 9 were constructed.

Differentiating this function with respect to time we obtain

$$\frac{dA}{dt} = \frac{b}{t}, \quad (8)$$

so that the rate of increase in area falls off in time. This function (7), however, goes on increasing to infinity, whereas the area of a single leaf reaches a maximum limit, so that the function is merely empirical, and holds only over a limited range of time.

Increase in Total Leaf Surface under Artificial Light.

In Fig. 10 are represented the curves of total increase in leaf surface for the average plant under 2 lamps and 1 lamp respectively. Obviously the process differs from that in the case of plants under natural illumination in

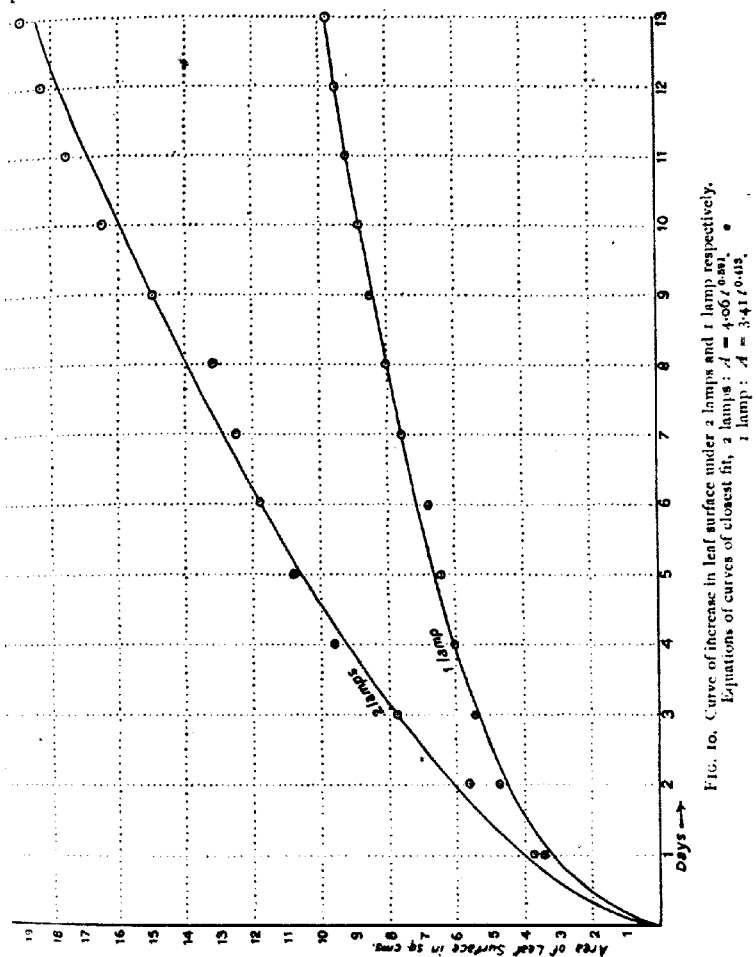


FIG. 10. Curve of increase in leaf surface under 2 lamps and 1 lamp respectively. Equations of curves of closest fit, 2 lamps: $A = 4.067 e^{0.41t}$, 1 lamp: $A = 3.417 e^{0.41t}$.

March and June, for the curves above are seen to be concave to the time axis, so that the leaf area is reaching a limit. It will be shown below how these results can be brought into line with those under natural illumination.

DISCUSSION OF THE RESULTS.

The growth in area of leaf surface. The curves of increase in area of the total leaf surface, obtained by plotting the daily leaf area data for March and June, are very approximately represented by a function of the form

$$A = ae^{rt}, \quad (9)$$

where A is area at any time, t after germination, a , r are constants. Differentiating this function with respect to time gives the relation between rate of increase in area and time. This operation gives

$$\frac{dA}{dt} = rA, \quad (10)$$

i.e. the rate of increase of leaf surface at any time is determined first by the area of leaf surface already present, and secondly by the value of the constant r .

If relation (9) were strictly true the leaf surface should go on increasing in a geometric ratio indefinitely, which certainly is not the case with annual plants, though taking the year as a time unit perennial plants may approximate to such a condition. The expression given above is therefore limited in its application to early stages in growth.

The curve of increase in area of the leaf surface throughout a growing season has been determined roughly by Kreuzler and his co-workers for a number of crop plants, and is of the ubiquitous **S** form. Thus two facts are clear from the author's and Kreuzler's results: (1) during the early stages of growth the leaf surface increases in geometric ratio; (2) the rate of increase gradually declines and finally becomes zero. Comparing the Cheshunt results for increase in area of total leaf surface during March and June, we find that the value of r , or what might be termed the 'rate of interest',¹ is 0.159 and 0.196 in the two cases. It is clear, therefore, that the magnitude of r depends on external factors.

It has already been pointed out that the 'average leaf areas' in the Cheshunt experiments are proportional to the total radiation per unit area during the period of the experiments, i.e. proportional to the product of light intensity and light duration, which must accordingly regulate the rate of increase.

Now these factors are varying during the periods of the experiments, particularly so in March, at which time the altitude of the sun goes through its period of most rapid increase, and hence it is not surprising that the curve of closest fit, calculated on the assumption that r is a constant, falls short of the experimental values for leaf area from the 19th to 24th days of the March experiment, and that for June the fit is much closer. Hence we

¹ A similar term r has been introduced by V. H. Blackman as a measure of the rate of production of dry matter, and the name 'efficiency index of dry weight production' was suggested. By analogy, this term r might be called the 'efficiency index' of leaf-surface increase.

are forced to the conclusion that r itself is a variable depending in magnitude on the external conditions.

Theoretically, then, the equation of increase in total area with time should be of the form

$$A_n = ae^{(r_1 + r_2 + r_3 + \dots + r_n)t}, \quad (11)$$

where r_1, r_2, r_3 , &c., are rates characteristic of successive days according to the total amount of light for each day. In practice, however, the whole problem is complicated by the changing weather conditions, so that we have no means of determining the values of r from the complex results. In spite, however, of the fact that the values of r for successive days are unknown, it is certain that there is a general trend of increase in the value of r from December 21 to June 21, and after this date by analogy the value of r should again fall. By assuming that r falls off according to a linear law and applying successive decreasing values of r in a compound interest formula, a series of values for the area will be obtained, which when plotted give a curve of S form, such as is the complete curve of leaf area, as shown by Kreusler.

By a simple modification of the compound interest formula the curve of S form is thus produced.

Now it is the resemblance of this curve to that of an autocatalytic reaction¹ curve which led Brailsford Robertson to apply, with great success in certain cases, the formula of an autocatalytic reaction to growth phenomena. Both Robertson's fundamental assumption and his conclusion, however, have been called into question by Enriques, who has shown that the form of function used by Robertson is limited in its application to S-curves in which the point of inflexion is not far removed from the half period of the reaction. Enriques, moreover, shows that the function used by Robertson is only a special case of a general function derived by integration of a differential equation of form

$$\frac{dx}{dt} = a + bx + cx^2 + dx^3 + \dots$$

To quote his words: 'So ist es möglich zu sagen dass die benutzte Formel in keiner Weise eine privilegierte Stellung in bezug auf die Nachahmung zwischen den anderen von demselben Typus besitzt,' &c. Evidence has been brought forward in this paper to show that, at least in the early stages of growth, the increase in area of the leaf surface conforms with a compound interest law, and it has been indicated how an extension of the conception of this law will account for the form of the complete curve of growth of surface area. The compound interest law, even in its extended form, is only an empirical expression of the course of the phenomenon of surface growth,

¹ When speaking of an autocatalytic reaction the term is employed in the following sense, namely, a reaction in which one of the products acts as the catalyst, and in which the material consumed continually decreases in mass as the reaction proceeds.

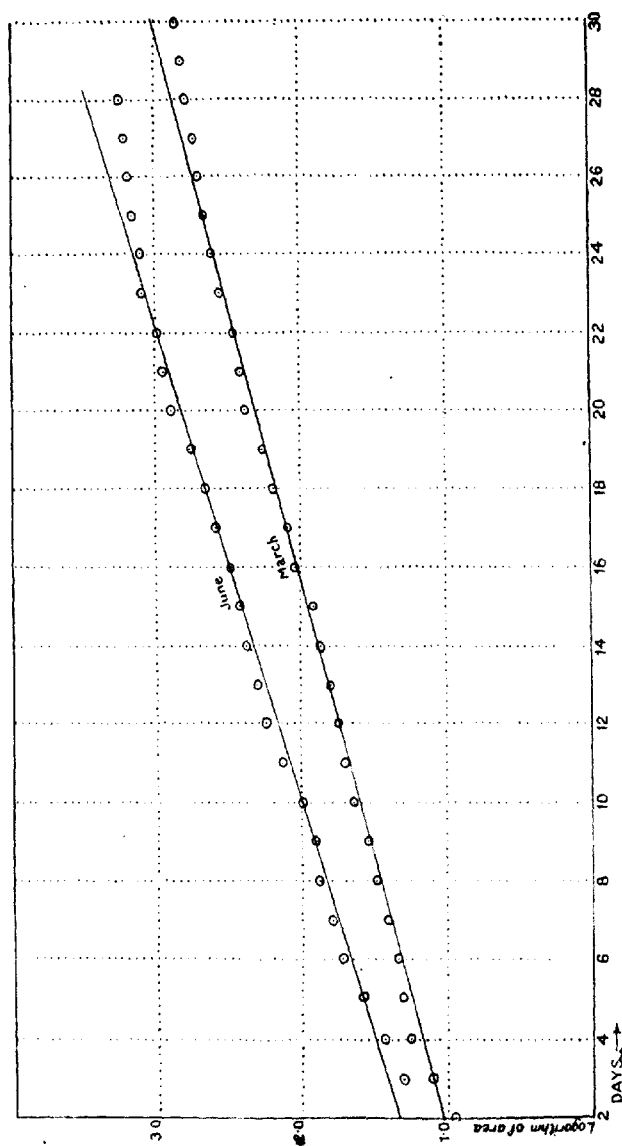


FIG. 11. Equations of curves of closest fit: March: $\log A = 0.0697 + 0.92$
 June: $\log A = 0.0577 + 1.15$

and will remain so until the relation of the change of r with time has been established, but as such it has the advantage over the conception of an autocatalytic reaction in that it is independent of any theory of the ultimate mechanism of growth."

Returning once more to the curves of increase in total leaf surface (Figs. 6 and 7), it is seen that the experimental values lie on a sinuous curve which fluctuates round the curve of closest fit. This is brought out more clearly in Fig. 11, where the logarithms of areas are plotted against time. This sinuosity is due to the fact that each leaf in its development goes through a grand period of growth, and that the number of developing leaves at each moment is small, so that periods of greater and lesser increase alternate. It is also seen that towards the end of the experiments the rate

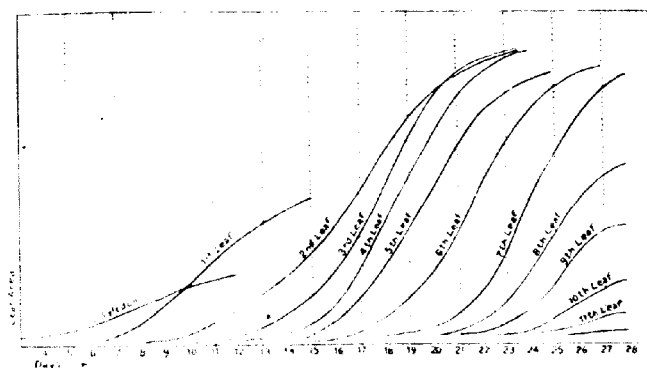


FIG. 12.

of increase markedly falls off. This can readily be accounted for by a study of Fig. 12, which shows for each day the actual areas of successive leaves from the cotyledons onwards, and it is evident that up to the eighth leaf the development is normal, but after this the later formed leaves go through their cycle of development in a shorter time and fail to attain the same maximum area as their predecessors. This inhibiting effect is due to the fact that the pots in which the plants are growing are more and more restricting root development. For this reason the last figures for leaf area are untrustworthy and fall short of the areas calculated from the compound interest formula. In corroboration of this conclusion it will be noticed that in March, where development is less rapid, the effect does not appear until the 28th day, while in June it is evident on the 24th.

The action of light as a limiting factor. The curve of increase in area of the total leaf surface of the 'average' plant in the December experiment (Fig. 13) is apparently of the same form as for March and June, but on

plotting the logarithms of the areas against time the curve obtained deviates rapidly from a straight line. By plotting the log areas against log time, however, a straight line is again obtained (Fig. 14).

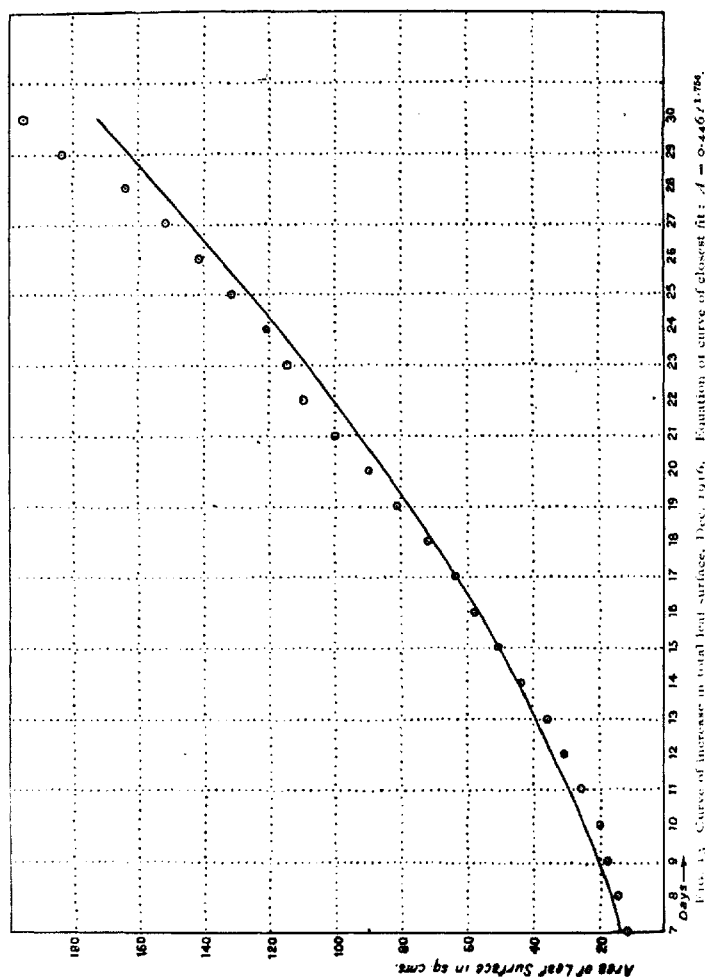


FIG. 13. Curve of increase in total leaf surface, Dec. 1916. Equation of curve of closest fit: $A = 0.462t^{0.894}$.

Here, then, we are not dealing with a compound interest phenomenon of the same type as before, but the expression of the course of this phenomenon is of the form

$$A = ae^{r \log t} = at^r. \quad (12)$$

Differentiating this with respect to time gives

$$\frac{dA}{dt} = \frac{rA}{t} \quad (13)$$

We see that the rate of increase in area still depends on the area already existing, but the constant r has been replaced by $\frac{r}{t}$. Hence in this case the rate of interest falls off continuously with time. In other words, we have a time factor at work, which continually tends to decrease the rate of expansion of the leaf surface of the plant. It is suggested that this detrimental factor is bound up in some way with the low intensity of light and the shortness of the day during the winter months, since the detrimental factor does not come into action in March and June, and can hardly be

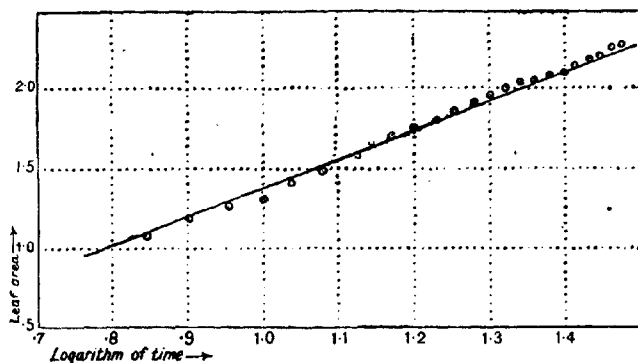


FIG. 14. Equation of curve of closest fit: $\log A = 1.756 \log t - 0.354$.

a temperature effect as the average temperature in the greenhouse in March was only 1.5°C . higher than in December.

The increase in total leaf area of a plant depends upon two factors: (1) the rate of production of successive leaves, (2) the rate of expansion of individual leaves. It is clear that the rate of production of successive leaves is a function of the activity of the vegetative point in which rudiments of the leaves are laid down, and one would expect that this would be regulated mainly by the temperature relations, though the rate of supply of available material for growth may act as a limiting factor. Thus plants which have large reserves of material will, when grown in the dark, produce rudimentary leaves until the reserves are exhausted; these leaves, however, usually fail to expand. On the other hand, evidence has been produced above to show that the area of the leaf surface is closely related to the intensity of the incident radiation.

Temperature, however, has another important effect on the plant, in

that it markedly affects the rate of respiration. Now during the winter months in the latitude of London the hours of darkness greatly preponderate over the hours of light (in December 8.5 hrs. light, 15.5 hrs. darkness), while the intensity of daylight is at a minimum; thus photosynthetic assimilation will not only be low, but the accumulation of material will be markedly reduced by respiration. With the high temperatures prevalent in greenhouses during the winter months this loss due to respiration may be considerable, so that the plant may be in a state of semi-starvation. The deleterious effects of high temperatures are seen in the experiments under artificial light.

Whether the detrimental factor discussed above is to be attributed to incipient starvation or to some other unknown factor concomitant with low intensity of illumination it is impossible, from the data so far collected, to decide. Moreover, there can be no doubt that light has, apart from its photosynthetic effect, a definite morphogenic action on the plant, so that the elucidation of the complex phenomenon awaits further investigations.

Fig. 15 shows the log time plotted against log area for plants grown under artificial light up to the 13th day, the time of repotting. Here again a straight line is obtained, so that, though the curves of total leaf-surface increase for plants grown in December (Fig. 13) and under artificial light (Fig. 10) appear so diverse, the same law is being followed under artificial illumination as under low intensity daylight.

The equations of the curves of total leaf-surface increase are as follows:

$$\text{December in daylight: } A = 0.45 t^{1.16},$$

$$\text{under two lamps: } A = 4.06 t^{0.698},$$

$$\text{" one lamp: } A = 3.41 t^{0.413}.$$

The different aspect of the curves is now clear, for when the index of t is greater than unity the curve will be convex to the time axis, when the index equals unity the curve becomes a straight line, and finally as the index falls below unity the curve is concave to the time axis.

In the case of plants grown under artificial illumination a detrimental factor is again seen to be at work.

Leaf-surface growth under artificial light. The intensities of the incident radiation in the two experiments with artificial illumination were as follows:

$$\text{With two lamps: } 0.216 \text{ cal. per cm.}^2 \text{ per min.}$$

$$\text{With one lamp: } 0.125 \text{ cal. per cm.}^2 \text{ per min.}$$

It will be seen in Table V that these intensities are approximately equal to the average intensities of radiation recorded at Cheshunt during the June and March experiments respectively.

The proportion of infra-red radiation to total radiation was, however, greater than in sunlight, so that the intensities as they are stated above are

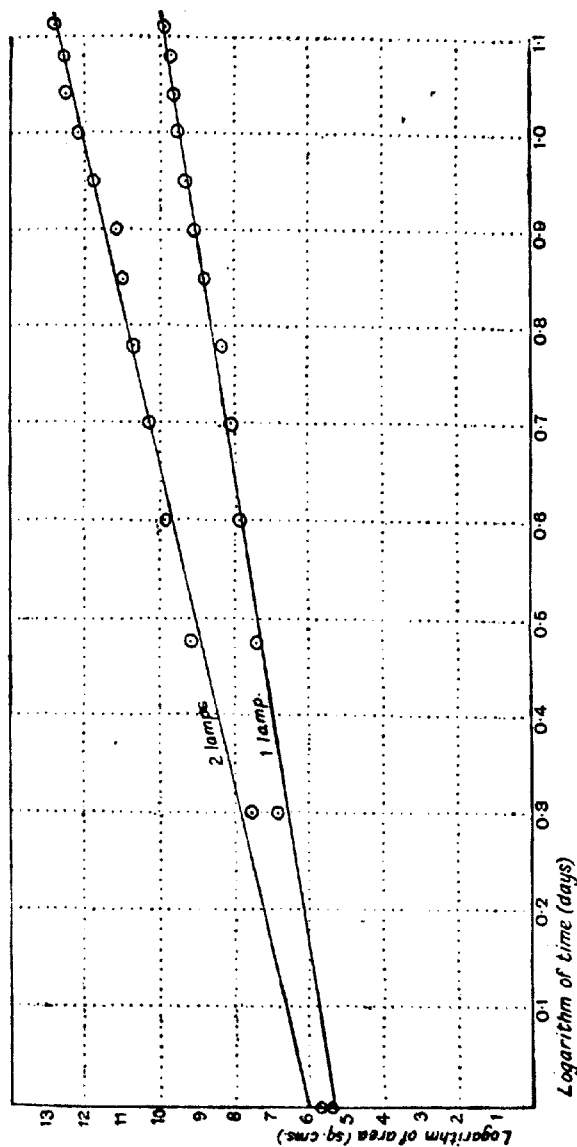


FIG. 15. Equations of curve of closest fit, 2 lamps: $\log A = 0.502 \log t + 0.609$,
1 lamp: $\log A = 0.413 \log t + 0.533$.

not comparable with those in Table V. The radiant efficiency of the lamps used was taken to be 5 per cent., which indicates that the proportion of infra-red radiant energy to total energy is of the order of 95 per cent., while for sunlight at sea-level the same proportion is of the order of 50 per cent., for a sun at zenith in the latitude of Washington. The figures for intensity of incident radiation must therefore be divided by 10 to give values comparable with those in Table V, and will then give values of the same order as the average intensity of radiation for December. From the foregoing considerations it seems reasonable to suppose that the temperature of the leaves of the plants under the lamps was considerably higher than the air temperature, for the plants were in still air, and in an atmosphere 75 per cent. saturated, both of which factors tend to check evaporation from the leaves. The air temperature was maintained at 35°C., and, moreover, the plants were exposed to this temperature during the whole period of growth. We may therefore, without much doubt, ascribe the action of the detrimental factor to the high temperature and low intensity of light radiation during these experiments.

Relation of light intensity to 'average leaf area'. In Table VIII the values for 'average leaf area' divided by total radiation for the Cheshunt set of experiments are stated. It is clear that for each set of experiments this value approaches constancy. We may therefore conclude that for comparable conditions the 'average leaf area' is determined by the product of intensity and of duration of light radiation.

TABLE VIII.

	<i>Total radiation per sq. cm. cals.</i>	<i>Average leaf area. sq. cms.</i>	<i>Average leaf area. Total radiation.</i>
Cheshunt—June . . .	7.077	516	0.073
March . . .	3.470	201	0.081
December . . .	8.49	59	0.069
Under 2 lamps . . .	8.086	17.6	0.0022
1 lamp . . .	4.682	10.2	0.0022

The part played by temperature in the increase of the leaf surface is not clear, and an investigation of this point is in hand. In the case of the Cheshunt experiments it appears that total radiation is the main controlling factor, while on the other hand the experiments with artificial light indicate that temperature relations may profoundly modify the action of light, if a super-optimal level is maintained. It would appear that for each light intensity there is a corresponding temperature at which the rate of increase of the total leaf surface attains a maximum, and if the temperature is raised above this level a detrimental factor comes into action tending to reduce the rate of increase of the leaf surface.

The method of investigation described makes it possible to determine

these optimal relations through a study of efficiency of plants under various conditions.

In concluding this part of the subject one may bring forward additional evidence for the compound interest law of leaf-surface increase. If the rate of increase of the leaf surface is determined by the leaf area already in existence, then the plant with large cotyledons should gain a start over the plant with smaller cotyledons, and this extra start should persist throughout the development of the plant.

For 100 cucumber seedlings the correlation coefficients have been worked out between

(1) the maximum area of cotyledons and maximum area of first leaf,

(2) the maximum area of cotyledons and the dry weight after 30 days.

These are as follows :

$$(1) r = +0.54,$$

$$(2) r = +0.36.$$

Furthermore, it was noticed by the author that plants with highest growth-rate later produced the largest crop of fruit, and in the Fourth Annual Report of the Cheshunt Experimental Station are published the growth-rate and crop weight for the average plants of eighteen batches of four plants each. From these data the author has worked out the correlation coefficient between growth-rate and crop weight, and finds that

$$r = +0.73.$$

The coefficient is high and certainly suggests that the whole future activity of the plant is correlated with the area of the cotyledons of the seedling.

Finally, it must be borne in mind that the results of this investigation apply only to plants grown under similar conditions, when the actions of limiting factors other than light intensity are excluded; and the results obtained cannot be applied directly to plants grown under other conditions of soil moisture and soil fertility.

SUMMARY.

A method is described for ascertaining the areas of leaves without detaching them from the plant. The errors of the method are discussed, and it is shown that a single determination of the total area of the leaf surface is significant to 5 per cent.

The growth in length and breadth and in area of leaves in daylight is shown to follow the same law as the growth in length of axial organs, displaying a grand period of growth. Under continuous electric light, however, the rate of increase falls off from the first measurement of area onwards.

The curves of increase in area and in linear dimensions for a single leaf in daylight are of S form, and can be fairly represented by the formula of an

autocatalytic reaction in which the material catalysed gradually decreases in amount as the reaction proceeds. A modification of this formula is introduced to represent the growth in area of single leaves under artificial light.

The increase in area of the total leaf surface of plants grown in daylight in March and June closely follows a compound interest law, the rate of increase at any time being proportional to the area existing at that time. For plants grown in December, however, the increase in total leaf area follows a more complex law, which may be represented by the function $A = ae^{r \log t} = at^r$; the rate of increase at any time is then given by the equation $\frac{dA}{dt} = \frac{rA}{t}$, showing that the rate of increase is still proportional to

the leaf area already extant, but tends to fall off with time, owing to the action of a detrimental factor. In the case of plants grown under artificial light, the increase in total leaf area is found to follow the same law as for plants grown in full daylight in December, and also shows the action of a detrimental time factor. It is suggested that the detrimental factor in this case may have been due to the high temperature maintained during the experiment.

It is shown that under comparable conditions the 'average leaf area' is determined by the product of intensity and duration of light radiation.

In support of the compound interest law of leaf-surface growth coefficients are quoted for correlations between

- (1) maximum area of cotyledons and maximum area of 1st leaf:

$$r = +0.54;$$

- (2) maximum area of cotyledons and dry weight of plant after 30 days:

$$r = +0.36;$$

- (3) growth-rate and crop weight:

$$r = +0.7.$$

In conclusion, the author wishes to record his thanks to Prof. V. H. Blackman for his unfailing inspiration and continual interest in the progress of the work; to Mr. A. B. Lister, secretary to the Experimental Station, Cheshunt, for many facilities for the work; and to Miss H. Marchant, laboratory assistant of the Experimental Station, for assistance in collecting data under very trying conditions.

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On the Occurrence in Britain of the Ascigerous Stage of a 'Brown Rot' Fungus.

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With Plates VI and VII.

ALTHOUGH the Brown Rot diseases of fruit trees have been under review for over a hundred years, and have attracted the attention of numerous workers because of their economic importance, the systematic relationship of the fungi concerned is still a debatable subject. This is chiefly owing to the fact that the ascigerous forms are met with comparatively rarely, especially in Europe, with the result that observations and descriptions have been chiefly confined to the conidial (*Monilia*) forms. The variation in the morphological characters of these *Monilias* in response to changes in the environmental conditions has created apparent discrepancies in such descriptions and has led to some confusion. This variation is well shown by *Monilia cinerea*, Bon., the grey fungus commonly found on plums and cherries, particularly with respect to the size of its conidia; thus the average size of the conidia produced on mummied fruit and dead twigs, in winter is about $11.5 \times 8 \mu$, while those conidia which develop on recently infected fruit in summer average about $17 \times 11 \mu$. That such variation is a specific character has been established experimentally (10) by cultivating pure line strains (each derived from a single conidium) and comparing their morphological characters when grown under varied conditions in the laboratory and on fruit trees in the open.

When Woronin (11), in 1888, published an account of the life-history of *Sclerotinia Vaccinii*, in which he showed that a *Monilia* found on the young shoots of the Cowberry, *Vaccinium Vitis-Idaea*, was the conidial form of a *Sclerotinia* which produced apothecia on the mummied fruit, it was surmised that the Brown Rot fungi *Monilia fructigena* and *M. cinerea* were also to be referred to that ascomycetous genus, and Schröter (8) in 1893 included them in his Kryptogamen-Flora as *Sclerotinia fructigena* and *S. cinerea*, although the corresponding ascigerous forms had not then been discovered. In 1902, however, Norton (5) found apothecia

developing from mummied peaches lying on the ground in a Maryland orchard and, assuming that they were the ascigerous fructifications of *Monilia fructigena*, Pers., named the fungus *Sclerotinia fructigena*. Since that date others have also found and commented on the *Sclerotinia* occurring in North America on peaches and plums; in the earlier records, e.g. those by Dandeno (3), Reade (7), and Pollock (6), Norton's naming was adopted, but the more recent papers by Matheny (4) and Bartram (2) lend support to the conclusion arrived at by Aderhold and Ruhland (1), who examined some of Norton's specimens, that the Brown Rot fungus commonly found on the stone fruit in America is not *Sclerotinia fructigena* but *S. cinerea*.

Aderhold and Ruhland (1) announced the discovery in Europe of apothecia on mummified apples and apricots, and in an interesting paper which appeared in 1905 gave descriptions of three apothecial forms under the names *Sclerotinia fructigena*, *S. laxa*, and *S. cinerea*. In 1912 Westerdijk (9) found a *Sclerotinia* on mummied cherries which did not quite conform to the description of any of the three species as defined by Aderhold and Ruhland; it was probably a Brown Rot *Sclerotinia*, but its genetic connexion with a *Monilia* stage was not traced. Until the present year these appear to be the only European records of *Sclerotinia* fructifications associated with conidial forms of the *Monilia fructigena* type, i.e. those in which the conidial fructifications consist of conidiophores (usually forming dense pulvinate tufts) bearing branched chains of ellipsoid or lemon-shaped conidia without disjunctors.

The descriptions given by Aderhold and Ruhland, and the conclusions they arrived at, have been generally accepted except by those who recognize but one species, viz. *Sclerotinia fructigena*, as responsible for the Brown Rot disease. Aderhold and Ruhland obtained cultures from the ascospores of the form which they found on apples, and conidial fructifications were produced conforming to descriptions of *Monilia fructigena*, Pers., thus justifying Schröter's adoption of the name *Sclerotinia fructigena* for this fungus.

Their conclusions with respect to *S. laxa* and *S. cinerea* are open to criticism however. In the first place, the specimens they named *Sclerotinia cinerea* were *preserved* material sent to them from America by Norton. Now it has been recently pointed out (10) that the Brown Rot fungus of America, although practically indistinguishable in its conidial stage from the grey *Monilia* occurring on plums and cherries in Europe, differs from the latter in certain cultural characters, and Aderhold and Ruhland's assumption that the American *Sclerotinia* is a stage in the life-history of the European *Monilia cinerea*, Bon., is untenable; moreover, a more recent examination of *fresh* material by several American mycologists, e.g. Pollock (6), Reade (7), Matheny (4), and Bartram (2), has shown that the dimensions of the asci and ascospores as determined by Aderhold

and Ruhland for *Sclerotinia cinerea* are too low for the American *Sclerotinia*. Again, Aderhold and Ruhland quote the size of the conidia as the chief distinguishing character of the *Monilia* forms of *S. laxa* and *S. cinerea*. They find the average size of the conidia of *S. laxa* to be $16.1 \times 10.8 \mu$ and that of *S. cinerea* $13.8 \times 9.95 \mu$; the former, however, is approximately the average size of the summer conidia of *Monilia cinerea*, Bon., the latter rather higher than that of the winter conidia of the same fungus.

In order to throw further light on the relationship of these forms, attempts have been made by the writer to induce the development of apothecia by exposing mummied fruit on the ground and in pots of soil in the open. These attempts at first met with no success, as the 'mummies' gradually decayed, leaving only the stones and cores. Early in the present year, however, apothecia were found on mummied plums; these were naturally infected fruit which had been collected during the winter of 1918-19, and in January, 1919, were placed in an ordinary flower-pot, which was then exposed on the ground in the open and left undisturbed for over a year. In the middle of March, 1920, the contents of the pot were examined, and it was found that, although the great majority of the plums had decayed, leaving only the bare stones, in the débris at the bottom of the pot a few black, wrinkled, skin-like bodies remained, one of which still enclosed a plum-stone, and growing out of them were apothecia in various stages of development. On one of the 'mummies' as many as twelve sporophores were counted.

DESCRIPTION OF THE *SCLEROTINIA* FOUND ON MUMMIED PLUMS.

The sporophores arose from a thin, wrinkled, sclerotium-like body (enclosing a plum-stone or taking the general shape of the stone which had fallen out), 0.3 to 0.5 mm. in thickness, and consisting of closely interwoven hyphae, together with disintegrated particles of the fruit; the outer and inner surfaces of the sclerotium were black, but the internal hyphae were hyaline.

Each apothecium was stipitate, the stipes measuring from 0.4 to 2.8 cm. and in some instances bearing rhizoids towards the base. The lower portion of the stipes was dark brown, almost black; the upper light brown, merging into the darker outer surface of the receptacle. The young sporophores were claviform, but the older ones had expanded at the apex and become at first crateriform, later plane (but usually with a depressed centre), and finally convex, and often split at the margin to form lobes. The apothecia examined were, when fully expanded, from 4 to 9 mm. in diameter; the hymenium was brown at first, but later became more or less pruinose, and at maturity grey or almost white. The asci were cylindrical, narrowed below, rounded above, and when treated with iodine each showed a bluish 'pore' at the apex; each ascus contained eight spores.

Microscopic examination of an apothecium which is liberating spores shows that when the ascospores are fully developed they are situated in the upper half of the ascus, at first in a single row (monostichous) and usually with their long axes oblique to the axis of the ascus, but shortly before they are expelled they become more or less distichous nearer the apex of the ascus, which meanwhile increases in size and becomes vacuolate, a single vacuole finally extending from the apex almost to the base. After discharging its spores the ascus contracts, and its wall shows minute striae; an ascus which measured $192 \times 12 \mu$ immediately before its spores were set free contracted to $162 \times 9 \mu$ after dehiscence (Fig. 5, *d* and *e*).

The ascospores are non-septate (a few spores with a single median septum were seen on one occasion), variable in size and shape, usually irregularly ovoid, some broadly ovoid, others elongate, sometimes flattened on one side; the ends are generally rounded, but often narrowed and almost pointed, occasionally one end mucronate (Fig. 7).

Paraphyses are present; they are approximately the same length as the asci before the latter elongate to discharge their spores, $2-2.5 \mu$ in diameter, and are either simple or bear from one to three branches; they are usually very slightly dilated towards the apex, but in some cases the tip was distinctly swollen (Fig. 6).

Since the dimensions of asci and ascospores are usually quoted as characters of primary importance in distinguishing the apothecial forms found on stone-fruit, careful measurements were made of asci and ascospores of three of the apothecia found on plums and the ascospores of a fourth. The first apothecium examined was probably not quite mature, for although the spores were fully formed in many of the asci and were readily set free on tearing out a portion of the hymenium in water, no asci with distichous spores (as seen later in older specimens) were observed; the second and third had asci with monostichous and others with distichous spores, as well as a number which had discharged their spores, while the fourth apothecium had become dry after reaching maturity, and when examined, after soaking in water, the asci were found to be disintegrated, though numerous spores were to be obtained.

The results obtained are given in the following table:

DIMENSIONS OF THE ASCI OF THE PLUM *SCLEROTINIA*.

Apothecium.	Asci with monostichous spores.			Asci with distichous spores.		
	Number of asci measured.	Range of variation.	Average size.	Number of asci measured.	Range of variation.	Average size.
No. 1	25	$124-163 \times 7.5-10.5$	143.6×8.8	None present	—	—
2	25	$128-170 \times 8.0-10.0$	151.0×8.9	10	$164-198 \times 11-13.5$	184.4×12.5
3	25	$150-188 \times 7.5-9.5$	168.0×8.4	30	$162-226 \times 10-12.5$	191.6×10.5
Result	75	$124-188 \times 7.5-10.5$	153.4×8.7	40	$162-226 \times 10-13.5$	192.0×11.5

DIMENSIONS OF THE ASCOSPORES.

Apothecium.	Number of spores measured.	Range of variation.	Average size.
No 1	100	11.0-18 x 5.0-7	13.6 x 6.1
2	100	8.5-17 x 4.5-8	12.2 x 6.0
3	100	9.5-19 x 4.5-8	12.3 x 6.3
4	100	7.0-19 x 4.5-8.5	11.9 x 6.3
Result	400	7.0-19 x 4.5-8.5	12.5 x 6.2

CULTURAL AND INOCULATION EXPERIMENTS.

The ascospores germinated readily in distilled water and on prune-agar, and germ-tubes up to $250\ \mu$ in length developed within twenty-four hours at room temperature (18° - 20° C.); the germ-tubes produced in distilled water were about $3\ \mu$ in diameter, and few produced branches during the first twenty-four hours (Fig. 8), while on agar the germ-tubes were 6 - $7\ \mu$ in diameter, and many were branched (Fig. 9). At the end of forty-eight hours the germ-tubes had produced numerous branches, and those growing on the agar had, in some instances, grown to a length of 2 mm.

Cultures were obtained by isolating ascospores on prune-agar plates and transferring the uncontaminated sporelings to other plates when two days old; in this way pure line strains were obtained, each derived from a single ascospore. These cultures on prune-agar plates showed the zoned and lobed growth (characteristic of strains of *Monilia cinerea*, Bon., obtained from plums, cherries, and peach twigs in this country) which distinguish it from the American *Sclerotinia*.

On transferring a little of the mycelium from the plate cultures to sterilized potato in test-tubes, white hyphae grew out at first, but within five days, at room temperature, grey tufts of conidiophores bearing chains of conidia of the *Monilia cinerea* type developed. 100 conidia from each of three such cultures on potato, eight days old, were measured, and the following measurements obtained:

(1) $9.0 \times 6.24.0 \times 15.5\ \mu$	Average $16.4 \times 11.9\ \mu$
(2) $9.5 \times 7.24.5 \times 18.5\ \mu$	" $15.9 \times 11.9\ \mu$
(3) $9.0 \times 8.23.5 \times 17.5\ \mu$	" $15.2 \times 11.5\ \mu$

These results are similar to those given by strains derived from conidia taken from plums naturally infected with *Monilia cinerea*; thus for seven such strains, when cultivated on steamed potato, and the average dimensions of 100 conidia produced on the medium determined for each, the averages varied from $15.5 \times 11.5\ \mu$ to $16.0 \times 12.5\ \mu$ and $17.0 \times 12.0\ \mu$.¹

In order to confirm the evidence supplied by the cultural studies that the *Sclerotinia* and the grey *Monilia* found on plums and cherries are forms

¹ See *Annals of Appl. Biol.*, vol. iii, p. 179.

of the same fungus, inoculation experiments were carried out on trees in the open, using conidia obtained from cultures derived from an ascospore. Inoculations were made on plum flowers and fruit, cherries (fruit), and apple flowers.

(a) *Inoculation of Plum Flowers.*

The inoculations were made by placing conidia on the stigmas of fourteen flowers of Victoria plum trees in the College plantation; the early symptoms of Brown Rot infection, i.e. brown discoloration of the stigmas and styles, were evident within four days, control flowers showing no such discoloration at this stage; the ovary was killed in every case, and on the twelfth day after inoculation nine of the flowers had grey *Monilia* pustules on the styles, and in some cases pustules appeared on the pedicels and calyx. About this time a gale removed all the inoculated flowers, and the question as to whether the fungus can establish itself in the flowering spurs and twigs was not determined. The experiment shows, however, that the fungus is capable of killing plum flowers by infection through the stigmas.

Measurements were taken of 100 conidia taken from the pedicel of one of the infected flowers, and the dimensions found to be $10 \times 8.5 - 20 \times 14 \mu$, average $14.8 \times 10.8 \mu$. The dimensions of conidia taken from a naturally infected flower in another plantation were $9.5 \times 7.5 - 28 \times 18 \mu$, average $14.5 \times 10.6 \mu$.

(b) *Inoculation of Plums (fruit).*

Conidia were placed in punctures made with a sterilized needle; seven of the eight plums inoculated became infected, and within nine days bore many grey *Monilia* pustules, while in one instance infection had already extended to a plum in contact with one of those inoculated. Conidia taken from one of the plums had dimensions of $10 \times 8 - 22 \times 17 \mu$, average $17.5 \times 13.0 \mu$.

As a control experiment eight plums were similarly punctured but not inoculated; these did not become infected.

(c) *Inoculation of Cherries (fruit).*

Cherries similarly inoculated were also readily attacked; in one instance pustules appeared within four days, on the rest pustules were observed on the sixth day. As in the case of the plums, where a sound cherry was in contact with an inoculated one, it became infected at the point of contact. Conidia taken from one of the cherries had dimensions of $11.5 \times 9 - 22.5 \times 18 \mu$, average $17.6 \times 13.2 \mu$.

It has been shown (10) that the average size of the conidia of *Monilia cinerea* when growing on fruit in summer is about $17 \times 11 \mu$, and it will be seen that the above dimensions for the conidia of the ascosporic strain produced under similar conditions are of the same order.

(d) *Inoculation of Apple Flowers.*

Apple flowers (variety James Grieve) were inoculated on the stigmas with conidia of the plum *Sclerotinia*, and simultaneously on the same tree others were inoculated with a strain of *Monilia cinerea* isolated from a dead flowering spur of an apple tree. For each strain eight inflorescences were selected and two flowers were inoculated on each inflorescence; thus sixteen flowers were inoculated with the *Sclerotinia* strain and sixteen with the apple strain of *Monilia*.

Of the eight inflorescences inoculated with the apple *Monilia* seven were killed outright, with all their flowers and leaves, the wilting of the leaves (which indicates that the fungus has extended from the infected flowers into the axis of the inflorescence) being noticeable in from twelve to seventeen days after inoculation of the stigmas.

Of the eight inflorescences inoculated with the plum *Sclerotinia* strain the flowering axis was not invaded in any one instance, although the inoculated flowers themselves showed the early symptoms of infection by a browning of the stigmas and styles when no such discoloration was observable on control flowers; one of these inoculated flowers, however, developed into fruit, while the rest fell off.

The results obtained from the inoculation experiments offer no evidence that the mode of parasitism of the fungus is modified in any way by the interpolation of the ascigerous stage, and confirm the conclusions given in a previous paper (10) that *Monilia (Sclerotinia) cinerea* is represented in Britain by two 'biologic forms', one of which causes a 'Blossom Wilt and Canker Disease' of apple trees, whilst the other is unable to extend farther than the pedicel of the inoculated apple flower. From its mode of growth in cultures, its parasitic habit, and the morphology of its conidial stage when growing on plums and cherries, together with its inability to invade the flowering spurs of apple trees, the *Sclerotinia* found on plums is shown to be the ascigerous stage of *Monilia cinerea* forma *pruni* referred to in that paper.

A COMPARISON OF THE BROWN ROT SCLEROTINIAS.

The following table summarizes the results obtained with respect to dimensions of asci and ascospores of Brown Rot *Sclerotinias* as determined by various authors:

Author.	Fungus.	Asci.	Ascospores.
Norton	<i>S. fructigena</i>	45-60 x 3-4	6-7 x 3-3.5
Reade	<i>S. fructigena</i>	125-215 x 7-10	10-15 x 5-8
Pollock	<i>S. fructigena</i>	130-179 x 9.2-11.5	11.4-14.4 x 5-7
Matheny	<i>S. cinerea</i>	From apothecia on peaches 135-190 x 6.9-10.5 Mostly 163 x 8.9	10.5-14.5 x 5.2-7.5 Mostly 12.5 x 6
		From apothecia on plums 135-173 x 6.8-10.8 Mostly 151 x 9.4	9.3-14.2 x 5-7.4 Mostly 11.8 x 6.3
Bartram	<i>S. cinerea</i>	Average 150.4 x 8.8	Average 10.1 x 7.1
Aderhold and Ruhland	<i>S. fructigena</i>	120.0-180.0 x 9.0-12.0	11.0-12.5 x 5.6-6.8
	<i>S. laxa</i>	121.5-149.9 x 8.5-11.8	11.5-13.5 x 5.2-6.9
	<i>S. cinerea</i>	89.3-107.6 x 5.9-6.8	6.2-9.3 x 3.1-4.6
Westerdijk	<i>Sclerotinia</i> on cherries	158.4-171.6 x 7.9-8.5	13.2-16.8 x 4.3-5.2
The <i>Sclerotinia</i> found on plums at Wye		Asci with monostichous spores 124-188 x 7.5-10.5 Average 153.4 x 8.7 Asci with distichous spores 162-226 x 10-13.5 Average 192 x 11.25	7-19 x 4.5-8.5 Average 12.5 x 6.2

With regard to the foregoing nomenclature and dimensions the following points are to be observed. As already mentioned, Aderhold and Ruhland established the connexion between the apothecial form they named *Sclerotinia fructigena* and the conidial form *Monilia fructigena*, Pers.; the ascospores of this fungus are described and figured as having pointed ends ('sporideis . . . ovato-fusoideis, utrinque acutis'). For their description of *S. cinerea* they used preserved material sent by Norton, and found that the ascospores had rounded ends: Reade examined fresh apothecia also obtained from Norton. It seems certain, therefore, that the earlier records of the *Sclerotinia* in America were not of *S. fructigena*, (Pers.) Schröter, and the more recent records show that the American form is one of which the conidial fructifications are indistinguishable from those of *Monilia cinerea*, Bon. It is to be noted that Reade's figures are higher than those of either Norton or Aderhold and Ruhland, and are of the same order as those obtained by Pollock, Matheny, and Bartram. Of thirteen strains of *Monilia* obtained by the present writer from various sources in North America, each has proved to be a fungus with a *Monilia* stage very similar to the grey *Monilia* common in Britain, but differing from the latter in its mode of growth when growing on agar, and in its more copious development of conidia when cultivated on artificially prepared culture media, while strains of *Monilia* isolated from mummied plums obtained from France and Holland have proved to be culturally similar to the form occurring in Britain. The American *Sclerotinia* would appear therefore to be either a distinct species or at least a form culturally distinct from the European *Monilia cinerea*, Bon.

There remain for consideration Aderhold and Ruhland's *Sclerotinia laxa* and Westerdijk's *Sclerotinia* on cherries; the asci and spores of the latter are greater than those found on *S. laxa*, but it will be seen that both

sets of dimensions are practically included in those given by the asci with monostichous spores of the *Sclerotinia* found on plums. The spores of *S. laxa* are described as 'fere semper monostichis, raro subirregulariter dispositis, utrinque obtusissimis, saepe 2-3 guttulis'; this general description of the spores conforms to Westerdijk's description and figures of the asci and spores of the cherry *Sclerotinia*. In neither case are asci with distichous spores mentioned, but as this is a condition which obtains only when the apothecia are quite ripe and liberating (or just about to liberate) spores, it might not have come under observation where suitable material was very limited.¹

The sclerotia from which *Sclerotinia laxa* developed were thicker (1 mm.) than those of the plum *Sclerotinia* (0.3-0.5 mm.), but this variation is probably merely due to a difference in the texture or size of the fruits.

Aderhold and Ruhland obtained cultures from the ascospores of the apricot *Sclerotinia* and reproduced the conidial stage; this they describe as appearing, to the naked eye, similar to *Monilia cinerea*; they found, however, that the conidia of the former were larger than those of the latter. Thus the dimensions of the conidia of *M. laxa* are given as $12.4-23.8 \times 9.3-15.5 \mu$ with an average of $16.1 \times 10.8 \mu$, those of *M. cinerea* as $9.3-14.5 \times 6.2-12.4$, the average size being $13.8 \times 9.95 \mu$. It will be seen from the inoculation experiments described in this paper that the dimensions obtained for the conidia of the plum *Sclerotinia* when growing on plums and cherries were in fact rather higher than those quoted for *Monilia laxa*.

Having regard to these facts, it would appear, therefore, that the *Sclerotinias* recorded for Europe as occurring on apricots, cherries, and plums show no essential morphological differences. To determine whether they are to be distinguished culturally, or biologically, with respect to their host plants will require further study. Aderhold and Ruhland suggest that there is a feeble specialization of parasitism, and that *S. laxa* is more or less confined to the apricot. Thus they had observed on one occasion that apricots were found severely attacked by Brown Rot, while neighbouring peaches and cherries were free from the disease; again, from their inoculation experiments, although they were able, with *S. laxa*, to infect the flowers not only of apricots but also of cherries, plums, and apples, they found that 'gegenüber *Sclerotinia laxa* die Pflaumenblüten sich relativ resistent erwiesen'. Though this evidence is suggestive it is not conclusive, and positive proof can only be obtained by parallel series of inoculations with strains of *Sclerotinia* from the various hosts.

¹ Aderhold and Ruhland appear to have obtained but one fully developed apothecium on apricots; they write, 'Von den beiden am 1. Mai Fruchtkörper zeigenden Aprikosen-Mumien trug die eine ein wohlentwickeltes und ein kleineres Apothecium, die andere 3 unvollkommen ausgebildete Apothecien'.

SUMMARY.

1. A *Sclerotinia* found on mummified plums at Wye in March 1920 has been shown, by cultural experiments in the laboratory and inoculations on fruit trees in the open, to be the apothecial stage of the grey *Monilia* commonly found on plums and cherries in Britain.
2. The fungus, of which a description is given, is to be referred to *Sclerotinia cinerea*, (Bon.) Schröter.
3. The dimensions of its asci and ascospores are, however, greater than those of the fungus described under that name by Aderhold and Ruhland; on the other hand, its morphological characters are not inconsistent with those of *Sclerotinia laxa* as defined by them.

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EXPLANATION OF PLATES VI AND VII.

Illustrating Mr. H. Wormald's paper on the Occurrence in Britain of the Ascigenous Stage of a 'Brown Rot' Fungus.

PLATE VI.

- Fig. 1. Two mummified plums bearing sporophores of *Sclerotinia cinerea*. Nat. size.
 Fig. 2. Isolated sporophores.
 Fig. 3. Four sporophores in situ. $\times 2\frac{1}{2}$. The uppermost one bears rhizoids at the base of the stipes.

Fig. 4. Culture, on prune-agar, grown at room temperature: the product of a single ascospore 2 days after isolating the spore (cf. Ann. of Bot., vol. xxxiv, No. 134, Plate IV, Fig. 5).

PLATE VII.

Fig. 5. Asci of *Sclerotinia cinerea*. $\times 500$. *a* and *b*. With monostichous spores. *c*. An ascus becoming vacuolate; spores not strictly monostichous. *d*. An ascus with a single large vacuole and distichous spores; condition a few seconds before the ascus dehisced. *e*. The same ascus shown in *d*, but after dehiscence. *f*. An ascus with three abnormally small spores.

Fig. 6. Types of paraphyses. $\times 500$.

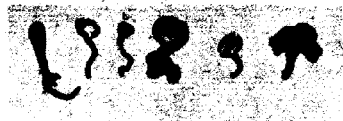
Fig. 7. Ascospores, showing variation in shape and size. $\times 1,000$.

Fig. 8. Ascospores germinating in distilled water: at end of 20 hours at room temperature (18° - 20° C.). $\times 500$.

Fig. 9. Ascospores germinating on prune-agar: at end of 20 hours at room temperature. $\times 500$.



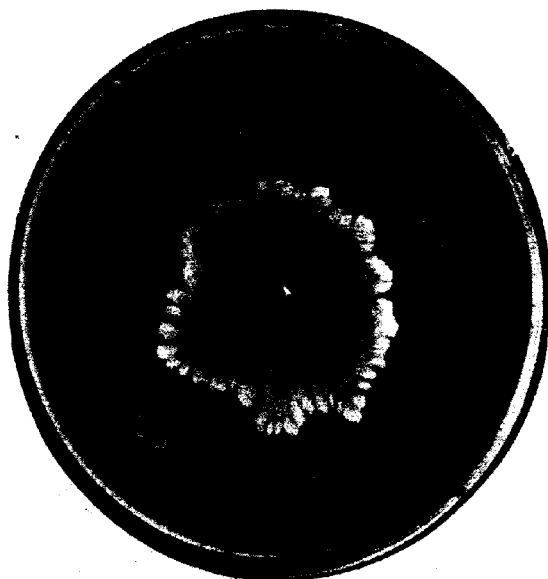
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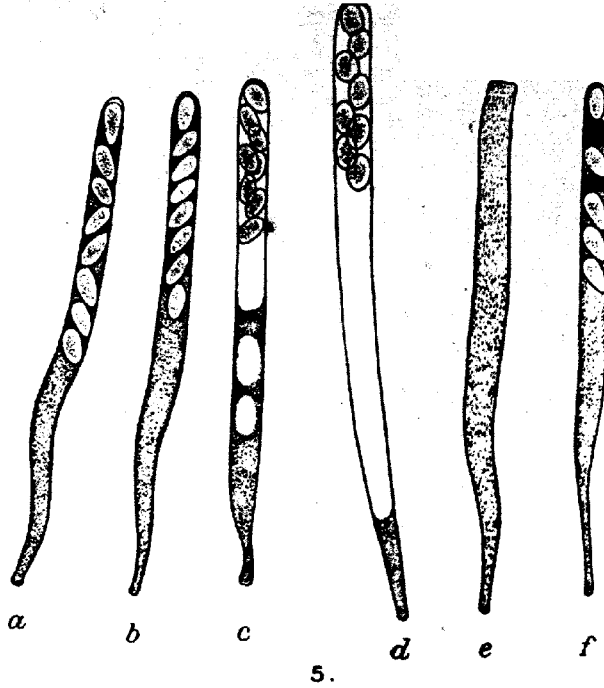


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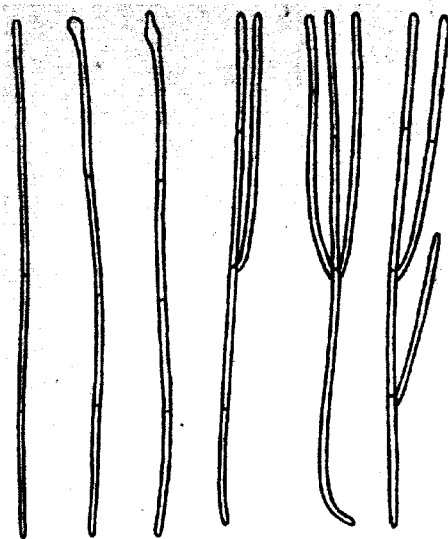


WORMALD-BROWN ROT.

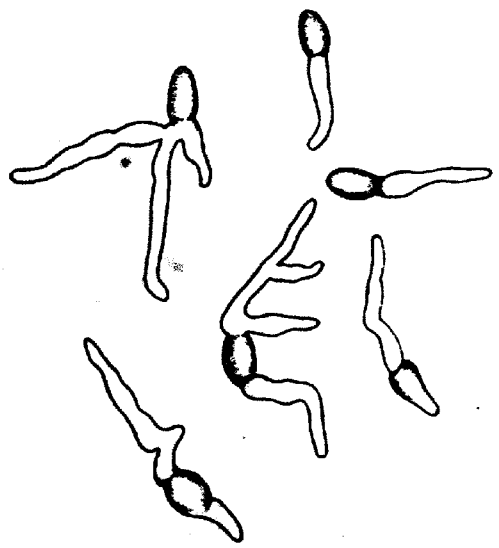
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WORMALD-BROWN ROT.



6.



9.

NOTES.

THE ISOLATION OF THE ORGANISM CAUSING CROWN GALL ON CHRYSANTHEMUM FRUTESCENS IN BRITAIN.—It is generally known that growths similar in character to those described in America as crown gall occur on plants of Paris Daisy (*Chrysanthemum frutescens*) grown in this country; but up to the present the identity has not been proved. The Ministry of Agriculture¹ regards the identity of the two diseases as established by the resemblance of the general features; the causal organism, however, so far as I am aware, has never been isolated from specimens of the diseased growths in Britain.

The object of this note is to record the isolation of the causal organism in pure culture; to describe its characters; and to establish its identity with the organism isolated by Erwin F. Smith and Townsend² in America.

The material necessary for the study of the galls was obtained from plants of the Paris Daisy grown in the nurseries near Manchester.

Portions of the galls from this source were used for infecting healthy plants of the Paris Daisy, and reinfection was found to take place very readily. The principal difficulty encountered in the isolation of the causal organism was that, when the usual method of sterilizing the outside of the gall with mercuric chloride solution was followed, the agar plates infected from the galls proved sterile. This sterility was probably brought about by the too rapid penetration of the sterilizing solution killing the organisms present. The organism was successfully isolated during August 1919 in the following way. A young, unsterilized, carefully washed gall was split open by means of a sterile knife, and the broken surface thus exposed was touched with a sterilized flattened platinum wire which was then used to make a parallel stroke culture on a + 10 bouillon-agar plate. Colonies of various organisms appeared, one of the most frequent showing a round, white, translucent, colonial growth. This organism was obtained in pure culture, and on using it to inoculate healthy plants of Paris Daisy galls regularly resulted. The same organism has been repeatedly re-isolated from the galls produced in this way. The characters of this organism, thus proved to be the causal organism of crown gall in the Paris Daisy in this country, have been carefully studied.

The organism is a bacillus, rod-shaped with rounded ends; measures unstained from young cultures on agar 1.2 to 1.5 μ by 0.5 to 0.6 μ ; paired rods 2.8 to 3.0 μ by 0.5 to 0.6 μ ; motile by means of one, but occasionally two or three, polar flagella. Neither spores nor capsules observed. Stains readily with the usual stains, e.g. gentian violet, Loeffler's methylene blue, carbol fuchsin, and carbol thionin. It is Gram-negative. Pseudozoogloea found in most liquid media.

On + 10 bouillon-agar, incubated at 18° C., colonies are visible after 48 hours; surface colonies small, round, entire, translucent, and white; maximum size 3 to 4 mm. On agar slopes growth moderate, white, filiform, smooth, raised, semi-transparent, becomes viscid with age, and is odourless. On + 10 bouillon-gelatin stab, growth

¹ Ministry of Agriculture Leaflet No. 245, Crown Gall; also Report on Insect and Fungus Pests, 1918, pp. 18 and 21.

² Smith, Erwin F., and Townsend, C. O.: Science, N.S., 1907, vol. xxv, pp. 671-3.

[Annals of Botany, Vol. XXXV. No. CXXXVII, January, 1921.]

takes place at the upper portion of stab, beaded or filiform; surface colony small, dirty white, no liquefaction on long standing. Gelatine plate colonies not characteristic; small, round, and whitish. Growth on potato good; white, wet-shining, slimy, spreading over lower portion of plug.

In + 10 bouillon stringy filaments suspended in a clear medium, best seen on shaking, probably due to a readily fragmenting pellicle.

Milk: coagulation delayed, separation of curd and whey by end of the first week, medium alkaline.

Cohn's solution: growth very scanty or absent.

Uschinsky's solution: no growth.

Thermal death-point between 44° C. and 46° C.

The result of a number of physiological tests may be summarized as follows:

Litmus peptone with one per cent. dextrose: only very slight acidity indicated by the end of the third week; no gas produced. Litmus peptone with one per cent. sucrose: similar results to that of dextrose. Peptone with one per cent. lactose: neither acid nor gas.

No reduction of nitrates. No production of indol. Diastatic activity feeble. Neither acid nor gas produced in bouillon-glycerine.

By the courtesy of Dr. Erwin F. Smith, to whom I desire to express my indebtedness, cultures were obtained of *Bacterium tumefaciens*, the causal organism of crown gall in America. When inoculations were made with these cultures typical galls were produced, and as regards the characters enumerated above, when compared throughout with the organism isolated in Britain, complete agreement was found. The conclusion that the two organisms are the same was thus proved.

During the detailed study of the two bacilli a few differences from the characters recorded by Dr. Erwin F. Smith and his collaborators were found. Thus the thermal death-point proved to lie between 44° C. and 46° C. compared with that of 51° C. recorded by Dr. Smith.¹ The acidity produced in dextrose and sucrose was so slight that the classification of the organism as an acid producer seems hardly justifiable.¹ No growth was observed to take place in Uschinsky's medium, while a 'scanty' growth has been recorded.¹

These differences apply both to the organism kindly sent by Dr. Erwin F. Smith and to the organism isolated in this laboratory, and in no way affect the conclusion just drawn that the two organisms are the same.

Summarizing briefly the results of this investigation, it will be seen that a bacillus has been isolated from crown gall occurring in Britain, and the characters of this prove identical with those of a type culture of *Bacterium tumefaciens*.

In conclusion, I wish to express my thanks to Dr. W. Robinson, who suggested this work, for the invaluable help and advice given during the course of this investigation.

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¹ Smith, Erwin F., Brown, Nellie A., Townsend, C. O.: *Crown Gall of Plants: its Cause and Remedy*. Bull. 213, Bureau Plant Industry, U.S. Dept. of Agriculture, 1911, p. 120, pp. 114-16.

A NEW METHOD OF PREPARING SECTIONS OF HARD VEGETABLE STRUCTURES.—In order to prepare sections of hard vegetable structures it is essential that some method should be devised by which the structure is not only embedded but softened, so that sections can be cut easily and smoothly. After various methods had been tried, the cellulose acetate method successfully used by Dr. Kernot for embedding and sectioning the fabric of aeroplane wings was used. It was discovered that this method not only embedded hard vegetable structures, but also softened them so that sections are easily obtained. It proved best to use cellulose acetate of French manufacture made from pure cellulose, as the viscosity is more uniform than in that of English manufacture, which is obtained from the cellulose of wood.

In the preliminary experiments pieces of oak and beech, cut into half-inch cubes, were passed through strengths of alcohol, then placed in pure acetone for two hours and finally into a 12 per cent. solution of cellulose acetate in acetone. There they were left for two months and excellent sections obtained. Further experiments showed that passage through alcohol was unnecessary. In the final experiments the pieces of wood were placed in water and the air removed from them, after which they were put into pure acetone for 1 to 2 hours and finally into the solution of cellulose acetate. It was found that the length of time of immersion in the solution of cellulose acetate necessary for softening the tissues varied with the hardness of the wood; the minimum time for soft woods being two days; for woods such as oak and beech at least six days are required. Experiments were tried with *sál* (*Shorea robusta*) and Pyingadu (*Xylia dolabriformis*), one of the Indian ironwoods, which is extremely hard. After fourteen days in the cellulose acetate solution it was possible to obtain transverse sections of these hard woods. The cellulose acetate solution is therefore capable of softening even the hardest wood in a relatively short time.

In order to stain sections—either hand or microtome—obtained by this method, it is necessary to wash them in pure acetone for 1 to 2 minutes to remove the cellulose acetate, wash in alcohol 1 to 2 minutes, and pass on to the stains selected. Various staining methods for cell-walls—such as aniline chloride, methylene blue, and Congo red, ammoniacal fuchsin and Kleinenberg's haematoxylin, &c.—were tried with success. A comparison with stained sections of untreated wood revealed no differences. Delicate tissues in the wood and hyphae of fungi infecting the wood also stain well and are unaffected by the treatment.

A satisfactory method of preparing sections of hard vegetable structures is therefore supplied by the use of a 12 per cent. solution of cellulose acetate in pure acetone for softening and embedding.

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The Gametophyte and Embryo of *Botrychium obliquum*, Mühl.

BY

DOUGLAS HOUGHTON CAMPBELL,

Stanford University, California.

With Plate VIII and eleven Figures in the Text

THE genus *Botrychium* comprises about thirty-five species,¹ of which about half occur in the United States. Most of the species are found in temperate regions, the few that occur within the tropics inhabiting the cooler mountain districts.

Our knowledge of the gametophyte and embryo is based mainly upon a study of two species, *B. Lunaria*, (L.) Sw.,² and *B. virginianum*, (L.) Sw.³ In 1905 Lyon⁴ published a brief account of the embryo of *B. obliquum*, Mühl., which was found to differ so much from the other species that he proposed to separate it as the type of a new genus, *Sceptridium*.

Dr. Lyon has very generously turned over to the writer his collection of gametophytes of *B. obliquum*, and also a large series of sections which he had made, as well as a number of photographs. From Dr. Lyon's sections, supplemented by sections made by the writer, a fairly complete study was made of the development of the reproductive organs and embryo, and it was thus possible to examine in detail the differences between *B. obliquum* and the two other species whose development was known.

THE GAMETOPHYTE.

The structure of the gametophyte of *B. obliquum* is much like that of the other species that have been investigated. The gametophyte is a subterranean, tuberous body, somewhat flattened horizontally and having a median, longitudinal, dorsal ridge upon which the antheridia are produced. The mature gametophyte (Pl. VIII, Figs. 3, 4; Text-fig. 1) is about 3 to 6 mm.

¹ Christensen, C.: *Index Filicum*, 1906.

² Hofmeister: *The Higher Cryptogamia*. Ray Society, 1862. Bruchmann, H.: *Flora*, xevi, 1903, 1906.

³ Jeffrey, E. C.: *Proc. Canad. Instit.*, 1898. Campbell, D. H.: *The Eusporangiatae*. Carnegie Institution Publication, No. 140, 1911.

⁴ Lyon, H. L.: *Bot. Gaz.*, xl, 455, 1905.

[*Annals of Botany*, Vol. XXXV. No. CXXXVIII. April, 1921.]

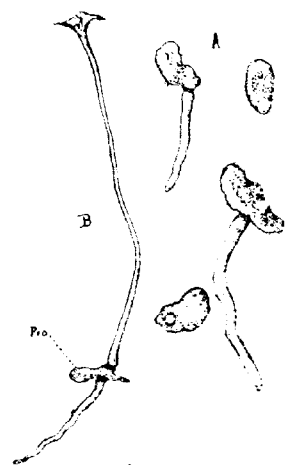
length and about half as wide. In size it is intermediate between *B. Lunaria* and *B. virginianum*.

The youngest specimens available (Pl. VIII, Figs. 1, 2) were broadly oval in outline, $\frac{3}{4}$ to $1\frac{1}{2}$ mm. in length, and the antheridial ridge had not yet developed. These young specimens had numerous long rhizoids, which are much less conspicuous in the more advanced stages.

As the gametophyte develops, it becomes relatively narrower, and often less regular in outline, and a more or less conspicuous median ridge is formed, upon which are borne the antheridia (Fig. 3). These begin to develop before the archegonia make their appearance. The latter usually occur close to the flanks of the antheridial ridge, but not upon it.

A longitudinal section of the young gametophyte shows the apex to be occupied by a group of meristematic cells, but it is not certain that there is a definite single apical cell, although the cell, *x*, in Fig. 8 may be such a single initial cell.

As in other Ophioglossaceae, there is present an endophytic fungus which occupies a large part of the inner tissue of the gametophyte, but is absent from the meristematic region. In the older gametophyte it does not invade the tissue immediately adjacent to the reproductive organs (Fig. 12), and a section of the gametophyte shows the dorsal region nearly or quite free from the fungus, which is abundantly developed in the ventral region. In the infested area the fungus hyphae fill the cell cavity, but the nucleus remains



TEXT-FIG. 1. A. Gametophytes of *Botrychium obliquum*, Mühl., some with attached sporophytes. \times about 3. B. Young sporophyte with expanded cotyledon, attached to the gametophyte. Pro, natural size. From photograph by Dr. H. L. Lyon.

intact, as has been noted in other cases (Fig. 10).

The endophyte of *B. obliquum* closely resembles that of *B. virginianum*, and, as in that species, there may be developed thick-walled spores (?)¹ (Fig. 10, *sp.*).

Jeffrey² thinks that the endophyte is related to the genera *Pythium* and *Completoaria*, but has not proposed a name for it.

No further study of the endophyte was made, as it does not seem to differ materially from that of *B. virginianum*.

¹ Campbell: loc. cit.

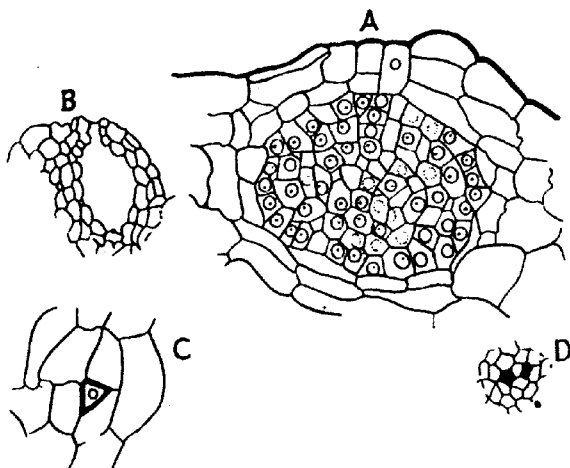
² Jeffrey: loc. cit.

THE ANTHERIDIUM.

The gametophyte is monoecious, but there is a good deal of difference in the relative number of antheridia and archegonia in different individuals.

The antheridia usually are produced in considerable numbers upon the median ridge, and, as usual, begin to develop before the archegonia. The latter as a rule form a row on either side of the antheridial ridge, but are not developed upon the ridge itself (Figs. 11, 12).

The development of the antheridium is very much like that of the other species (Figs. 13-15). The mother-cell divides first by a transverse wall into an outer, or primary, cover cell, and an inner cell from which the sperm cells finally develop.



TEXT-FIG. 2. A. Median section of an antheridium just before the final division of the spermatocytes. $\times 270$. B. Empty antheridium. $\times 90$. C. Surface view of antheridium with triangular opercular cell. $\times 270$. D. Surface view of antheridium with two opercular cells. $\times 90$.

The primary cover cell divides by vertical walls into several cells, in which horizontal walls are formed, so that there are two layers of cover cells. The horizontal wall is suppressed in one of the cover cells, however, and this becomes the opercular cell, where the antheridium opens. A second opercular cell is sometimes present (Text-fig. 2, D), but as a rule only one is formed. This opercular cell is destroyed at the time of dehiscence. In cross-section the opercular cell is generally four-sided, but sometimes it is triangular, as in *Ophioglossum* (Text-fig. 2, C).

The first division in the inner cell of the young antheridium may be either transverse or vertical, and is next followed by a wall at right

angles to the first one. The later divisions do not show any definite order, and the number of spermatocytes formed varies a good deal.

The material was not particularly favourable for a study of spermatogenesis, and, as there was nothing to indicate any deviation from what has been observed in other Ophioglossaceae, no attempt was made to study it in detail.

It may be assumed that, as in other pteridophytes, the body of the spermatozoid is derived mainly from the nucleus of the spermatocyte. The nucleus becomes an almost homogeneous, thickened band tapering towards the anterior end of the spermatozoid. In the later stages, the slender coiled blepharoblast, from which the numerous cilia arise, can be readily demonstrated, but the earlier stages of the blepharoblast were not studied. As the fully developed spermatozoid closely resembles that of *B. virginianum*, it may be assumed that the early stages of development are the same.

The mature spermatozoid (Fig. 18) is nearly twice as large as that of *B. virginianum*, but otherwise is very similar. In its large size it recalls *Ophioglossum*.¹ The walls of the ripe sperm cells stain very strongly, indicating that there has been a development of mucilaginous matter in the walls. These walls stain especially strongly when treated with Bismarck brown.

Of course it was not possible to study living spermatozoids, but a good many of the sections showed recently opened antheridia which contained some of the free spermatozoids which were pretty well fixed.

The earlier archegonia are formed in a line on either side of the antheridial ridge, but later they may develop behind the growing-point of the gametophyte at almost any part of the dorsal surface.

The earliest stages are hardly distinguishable from very young antheridia (Figs. 19, 20). The first wall is transverse and cuts off an outer from an inner cell. The former, by further divisions, forms the neck cells, the latter the egg and canal cells. As a rule no basal cell is present, but sometimes it looks as if such a cell might occasionally be formed.

The cover cell is usually rather shallow, but sometimes may equal in depth the inner cell (Text-fig. 3, A). As in all ferns, the first divisions in the cover cell are intersecting median vertical walls determining the four rows of neck cells.

By the time the first divisions in the cover cell are completed, the inner cell begins to grow up between the four neck cells, and as these elongate and undergo further divisions, there is formed a slender extension of the central cell which later is cut off from it as the neck canal cell. The separation of the canal cell from the central cell occurs at an unusually late period in *B. obliquum* when compared with the other Ophioglossaceae.

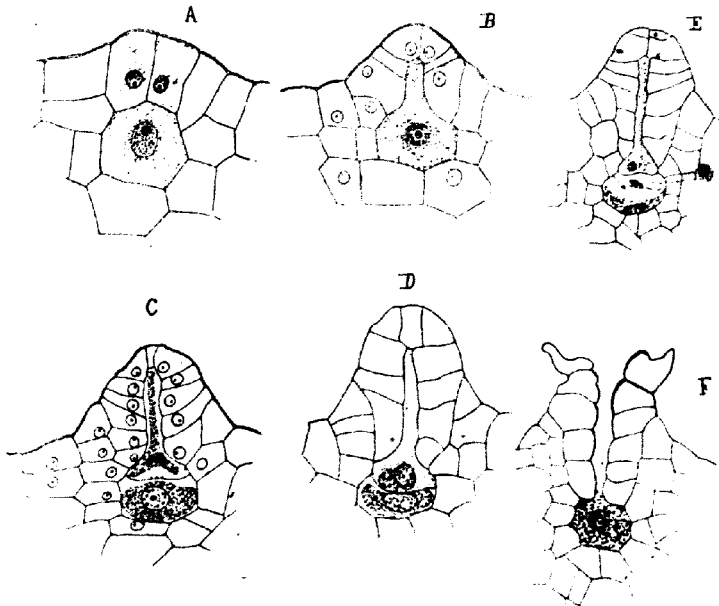
Each of the four primary neck cells divides by a series of oblique

¹ Campbell: loc. cit.

transverse walls into about six. Of these the upper half, approximately, extend above the surface of the gametophyte, the others lying below it (Text-fig. 3).

The nucleus of the neck canal cell later divides into two, but no cases were seen where a division wall had been formed, as is not unusual in *Ophioglossum pendulum*, but has not yet been demonstrated in *Botrychium*.

The presence of a ventral canal cell could not be demonstrated satis-



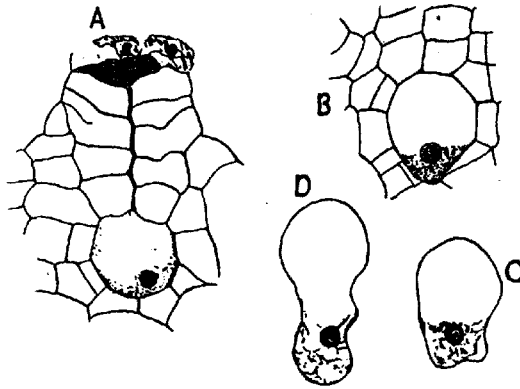
TEXT-FIG. 3. A. Young archegonium. $\times 320$. B-D. Older archegonia. $\times 210$. E. Mature archegonium, showing apparent ventral canal cell, v. $\times 240$. F. Open, but unfertilized, archegonium. $\times 240$.

factorily, although several preparations were secured which indicated that a ventral canal cell—or at least a nucleus representing this—was present. In Fig. 22 there is shown one such case, where in close contact with the nucleus of the central cell a second smaller nucleus is visible and a zone of somewhat denser cytoplasm, but no evident cell wall. In Text-fig. 3, E, another example is shown. In this instance there was apparently a small cell between the egg and the neck canal cell. The nucleus of this cell (?) was small and not very distinct.

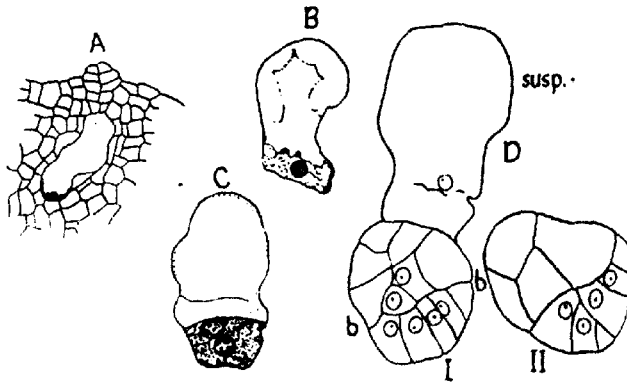
A similar difficulty in demonstrating a ventral canal cell has been experienced by the writer in his investigations of other *Ophioglossaceae*. It seems probable that in all of these the ventral canal cell is cut off very

soon before the opening of the archegonium and almost immediately ejected. It is also not unlikely that there may be no division wall formed and that the ventral canal cell is represented merely by a nucleus.

When the archegonium opens the terminal neck cells diverges widely, presenting a decidedly characteristic appearance (Text-fig. 3, F).



TEXT-FIG. 4. A. Recently fertilized archegonium containing unicellular embryo. $\times 270$. B-D. Older unicellular embryos. $\times 270$.



TEXT-FIG. 5. A. Archegonium containing much elongated unicellular embryo. $\times 100$. B. First division in the embryo. $\times 270$. C. An older stage. $\times 270$. D. Two sections of an older embryo. $\times 270$. I shows the large suspensor, *susp.*; b-b, basal wall.

In both *B. Lunaria* and *B. virginianum* a somewhat larger part of the neck is free than in *B. obliquum*. In this respect the latter shows a certain resemblance to *Ophioglossum* and the Marattiaceae.

THE EMBRYO.

The embryo of *B. obliquum* differs remarkably from that of either *B. Lunaria* or *B. virginianum*. Lyon¹ called attention to the presence of a conspicuous suspensor and a very different orientation of the cotyledon and root when compared with *B. virginianum*. He describes and figures the young sporophyte at about the time that the root emerges from the gametophyte, but he does not state just what is the relation of the organs of the young sporophyte to the early divisions of the embryo.

Bower² has figured several unicellular embryos from Dr. Lyon's preparations, showing the marked elongation of the embryo before the first cell division, but no later stages were found.

B. obliquum was the first fern in which a suspensor was demonstrated, but subsequently the writer discovered a suspensor in two members of the Marattiaceae, *Danaea*³ and *Macroglossum*.⁴ *Helminthostachys* has also been shown to possess a suspensor.⁵

The following account of the embryo of *B. obliquum* is based in part upon slides made by Dr. Lyon, and partly upon preparations made by the writer from prothallia and young sporophytes collected by Dr. Lyon.

Unicellular embryos are not uncommon, as several archegonia may be fertilized, and begin to form embryos; but the later stages are not so easily found, and it was not possible to secure as complete a series as might have been wished. However, the essential points in the development of the embryo were made out, and there is no question as to the way in which the young sporophyte develops.

As usual, the fertilized egg immediately forms a cell-wall, and grows until it completely fills the cavity of the venter. The unicellular embryo is at first globular in form, and contains only a small amount of granular cytoplasm in which is embedded the relatively small nucleus (Text-fig. 4, A). The lower part of the embryo next becomes slightly pointed, and this projection contains most of the granular contents of the cell, and the nucleus, which has become somewhat larger. The embryo now grows downward, as Bower has shown, and bores its way into the adjacent prothallial tissue. Sometimes it grows vertically downward, but more often its course is more or less oblique. At this stage it forms a somewhat irregular tube, whose apex is narrower than the basal portion within the venter of the archegonium. The nucleus and the surrounding granular cytoplasm, which has increased considerably in amount and shows a more or less evident acrolation, occupy the growing apex of the embryo-tube, which may be three times as long as wide before the first cell division occurs (Text-fig. 5, A).

¹ Loc. cit.

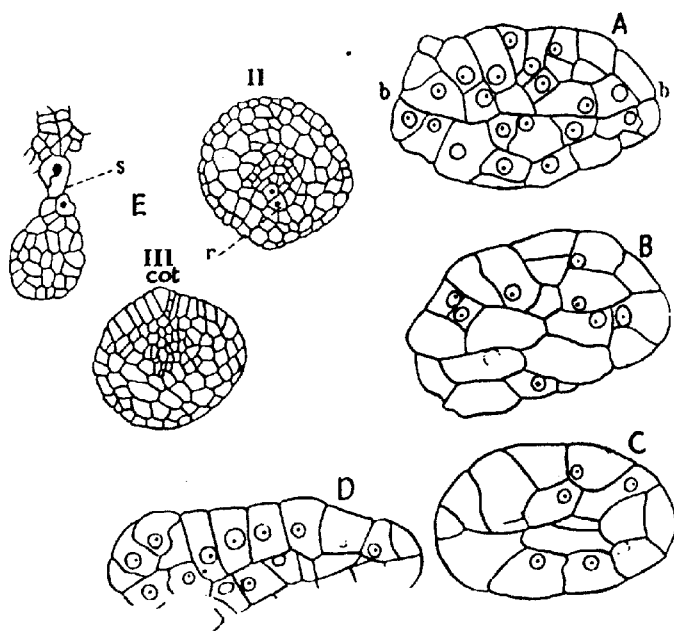
² Bower, F. O.: Origin of a Land Flora, Fig. 266.

³ Campbell: loc. cit.

⁴ Campbell: Ann. Bot., xxviii, 622, 1914.

⁵ Lang, W. H.: Ann. Bot., xxiv, 611, 1910.

Only a small number of the next stages were found, and these were not very well fixed and showed more or less shrinkage. Text-fig. 5, B, shows the first division in the embryo, by which the large suspensor cell is cut off from the terminal cell which is to form the embryo proper. This division is transverse, and is followed by a second division in the terminal cell which probably is to be considered as the basal wall, dividing the embryo into an epibasal and a hypobasal region. So far as could be judged, the direction of the basal wall is always approximately horizontal. There is probably some variation in the orientation of the basal wall with reference to the



TEXT-FIG. 6. A-D, Four of a series of sections of a young embryo. $\times 270$. E, Three similar sections of an older embryo in which the cotyledon and root are already evident. *s*, suspensor; *r*, root initial; *cot*, cotyledon. $\times 100$.

axis of the suspensor cell, which also is sometimes almost horizontal in position.

Text-fig. 5, D, shows two sections of a somewhat older embryo. The nearly globular embryo is attached to the large suspensor cell, and is probably cut in a nearly median plane. The epibasal region is occupied by a series of columnar cells, the central one of which is possibly the initial for the stem apex. Just below the basal wall is a triangular cell which suggests a root initial; but as no stages between this embryo and much

more advanced ones were found, the true nature of these apical (?) cells must for the present remain in doubt.

The next stages, which were a good deal more advanced, were remarkably like corresponding ones in the Marattiaceae, especially *Danaea*.

Text-fig. 6, A-D, shows four of a series cut vertically, showing plainly the basal wall (*b-b*). The embryo was broadly oval in outline, the major axis being transverse, as it is in corresponding stages of various Marattiaceae. Occupying the central portion of the epibasal region, there is a group of superficial columnar cells, which may mark the beginning of the cotyledon, but this is not at all certain. In this embryo there was nothing which could be interpreted as a root, and the whole of the hypobasal portion formed the large foot.

The next stage found (Text-fig. 6, E) was considerably older, and also strongly resembled the embryo of *Danaea*. The vertical axis of the embryo was now equal to the transverse, and the upper part was slightly pointed with what looked like a single apical cell marking the position of the cotyledon. The primary vascular bundle, which later extends through the cotyledon and root, is indicated by a group of actively dividing cells below the cotyledon apex.

The primary root in *B. obliquum* closely resembles in its origin that of *Ophioglossum* and the Marattiaceae. A single initial cell arises near the centre of the embryo, very near the basal wall (Text-fig. 6, E). In the embryo figured, the first segment has been cut off from the initial cell. It is difficult to say whether the root belongs to the epibasal or hypobasal region, as it is so near the centre of the embryo.

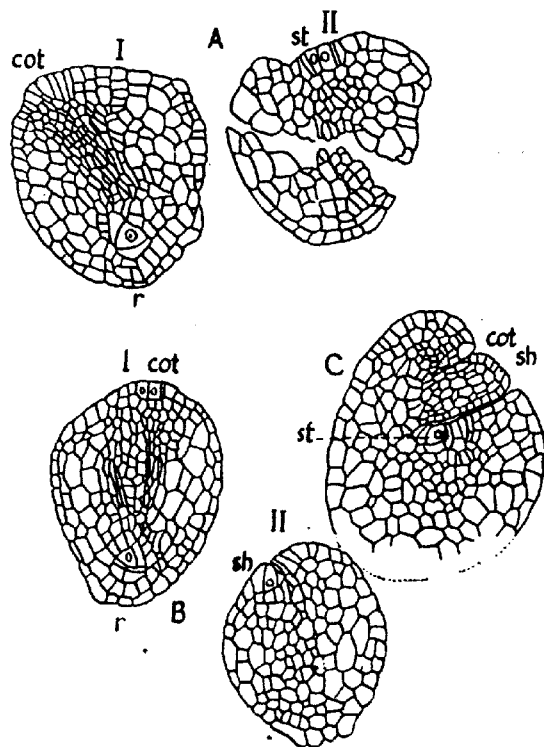
Text-fig. 7, A, shows two longitudinal sections of a more advanced stage in which the apical cell of the root is very conspicuous and the primary vascular bundle extends from the cotyledon into the root. The section passes through the apex of the cotyledon, which shows a very evident apical cell. The apical cell of the root is extremely conspicuous and closely resembles that of the roots of other Ophioglossaceae.

As in *Ophioglossum moluccanum* and the Marattiaceae, there is no question that the vascular system in *B. obliquum* begins as a single strand common to cotyledon and root and having no connexion with the stem apex. The latter (Text-fig. 7, A, II) at this stage consists of a small group of superficial cells, lying somewhat to one side of the centre of the flattened area forming the top of the embryo, which is somewhat top-shaped in outline, closely resembling similar stages in *Danaea*.¹ There is a single apical cell, which is somewhat larger than the neighbouring cells. In longitudinal section this cell is oblong in outline, with a broad, truncate base. In older stages, the lateral walls of the apical cell usually converge towards the top, which is thus narrower than the base.

¹ Campbell: *Eusporangiac*, Figs. 117-119.

This type of apical cell is found in most species of *Ophioglossum*, and in many Marattiaceae, but is unlike that of either *B. virginianum* or *B. Lunaria*.

Very soon after the cotyledon and stem apex are recognizable, the second leaf arises as a group of meristem cells close to the stem apex. The



TEXT-FIG. 7. A. Two longitudinal sections of an embryo, cut in the plane of the cotyledon. *cot*. The root, *r*, is very conspicuous. *st*., stem apex. $\times 100$. B. Two sections of a similar embryo, cut in a plane at right angles to that of A. I shows the leaf-traces of the cotyledon and second leaf. C. Somewhat oblique section of an older embryo showing the conspicuous sheath, *sh*., at the base of the cotyledon.

vascular bundle belonging to the second leaf is formed very early and joins the primary bundle extending through the cotyledon and root. The stem apex occupies the space between these bundles, but has no procambium tissue developed from it. The relation of the vascular bundles to the leaves and stem apex is best seen in a series of cross-sections (see Text-fig. 1c).

The cotyledon is provided with a very conspicuous basal sheath, which is not found in *B. virginianum*. At an early period there is active growth at the base of the cotyledon, which soon extends over the stem apex and second leaf, which lie in a narrow cleft formed by this overarching basal sheath of the cotyledon (Text-fig. 7, C). For some time the sheath is the most conspicuous part of the cotyledon, the apical portion of the leaf being relatively insignificant.

THE ROOT.

The characteristic tetrahedral apical cell of the root becomes established at an early period, and soon shows a regular segmentation. The root grows rapidly and emerges on the ventral surface of the gametophyte (Fig. 4). It is much the most conspicuous feature of the young sporophyte and reaches considerable size before the emergence of the cotyledon.

The root grows vertically downward through the tissues of the foot—or perhaps it would be more accurate to say it becomes incorporated with it, as the foot as such becomes quite unrecognizable, its tissues merging insensibly with those of the root, and its lower portion forms practically part of the root-cap, and it is ultimately destroyed as the root-cap is renewed from the segments of the apical cell. The axis of the root coincides with that of the cotyledon, so that almost from the first the bipolar character of the young sporophyte is established.

Some time after the root has broken through the ventral surface of the gametophyte, the cotyledon elongates and ruptures the dorsal surface, so that the position of the young sporophyte is very much like that of the Marattiaceae, or *Ophioglossum moluccanum* (Fig. 6; Text-fig. 1).

The apex of the cotyledon becomes differentiated into petiole and lamina, the latter being bent over, much as in *B. virginianum*. The young cotyledon shows a definite apical cell which appears narrowly triangular in longitudinal section.

The massive basal sheath extends over the stem apex, which, together with the second and third leaves, is completely enclosed within the sheath (Text-figs. 8, 9). A single strand of procambium extends through the petiole, but no vascular tissue develops in this sheath. The bundle in the petiole is continued downward into the root without a break, and it is quite impossible to determine the limits between leaf and root.

The second leaf develops in much the same way as the cotyledon, and its basal sheath envelops the stem apex and next younger leaf in exactly the same fashion. The details of the apical growth were not followed, but probably would not differ materially from the cotyledon.

The leaf-trace of the second leaf begins to form almost as soon as the leaf can be recognized, the same being true of the third leaf. The bundle

of the second leaf joins the primary strand of the cotyledon and root at a considerable distance below the level of the stem apex.

The first tracheary tissue in the primary bundle is formed at a point a little above the point of junction with the trace from the second leaf. The primary tracheary tissue consists of a bundle of short reticulate tracheides.



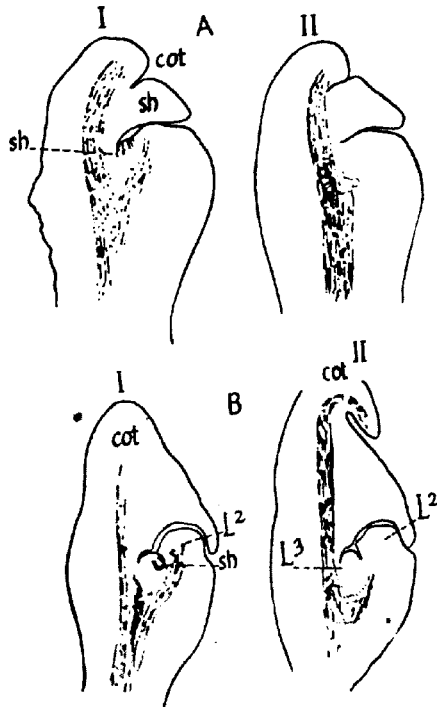
TEXT-FIG. 8. From a microphotograph of a young sporophyte just before the emergence of the cotyledon. *s.*, suspensor; *ar.*, archegonium. Photograph by Dr. H. L. Lyon.

From this point the development of permanent tissue in the bundle proceeds upward into the petiole and downward into the root. Shortly after the formation of the first tracheary tissue in the primary bundle, tracheides appear at the base of the trace from the second leaf, where it joins the primary bundle, and from this point the formation extends upward.

With the elongation of the cotyledon it soon breaks through the overlying tissues of the gametophyte and emerges upon its dorsal side. Then,

pushing through the soil, it appears above ground as a small ternate leaf, with a long slender petiole (Fig. 7).

The venation of the leaf-lobes is dichotomous, and suggests that the ternate form of the lamina is due to an unequal dichotomy such as can often be seen in many ferns in the early leaves intermediate between the



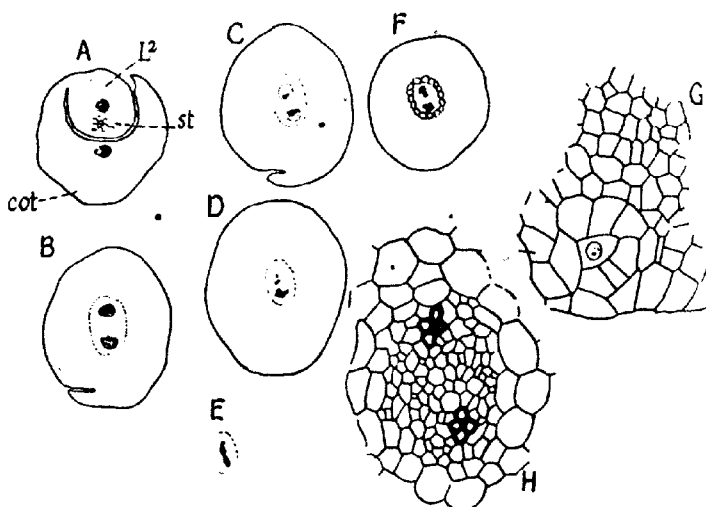
TEXT-FIG. 9. A. Two longitudinal sections of a young sporophyte, showing the arrangement of the vascular bundles. \times about 70. B. Two sections of a somewhat older sporophyte, showing second and third leaves, L^2 , L^3 .

dichotomously lobed cotyledon and the pinnate leaves of the older sporophyte.

The position of the young sporophyte, with reference to the gametophyte, is exactly the same as in the Marattiaceae, the bipolar sporophyte completely perforating the gametophyte, the cotyledon emerging above, the root below. It must be remembered, however, that in the Marattiaceae, as in the ordinary ferns, the archegonia are upon the ventral surface of the prothallium instead of upon the dorsal side, as is the case in *Botrychium*.

The arrangement of the vascular bundles can best be understood from a series of cross-sections. Text-fig. 10, A-F, shows several sections from such a series from a young sporophyte of about the same age as that shown in Text-fig. 8; A shows the section passing through the stem apex, *st.*, which with the second leaf is surrounded by the sheathing base of the cotyledon. The vascular bundle of the latter shows the first tracheides at the inner limit of the bundle. No tracheary tissue has yet been formed in the second leaf.

In sections taken farther down the two bundles approach, and finally



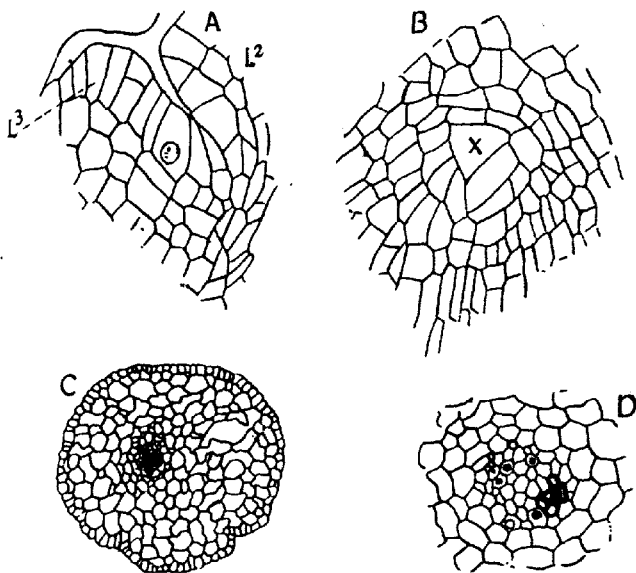
TEXT-FIG. 10. A-D. Four transverse sections of a young sporophyte showing the coalescence of the leaf-traces to form the axial stele. A passes through the stem apex, *st.*, *L*² youngest leaf. E. The stele, showing the complete coalescence of the xylems of the two leaf-traces. F. Section of the root, showing the endodermis and diarch bundle. G. Stem apex. $\times 270$. H. Root bundle. $\times 270$.

coalesce. While the two bundles maintain their identity for some time, they occupy a pretty well defined oval area, which, however, is not limited by a recognizable endodermis. After the coalescence of the bundles is completed, and the stele enters the root region, a definite endodermis of the typical form is easily seen (F, H).

Just before the two leaf-traces coalesce, the first tracheary tissue can be seen in the trace from the second leaf, and the xylems of the two leaf-traces form a continuous band at the junction of the two bundles (E). The two leaf-traces are now completely merged into a common 'stele', which is continued downward into the root. It is quite impossible to say where the foliar portion of the stele ends and that of the root begins.

The suspensor increases in size and becomes multicellular, but it is not quite clear whether this is entirely the result of division in the primary suspensor cell or whether the cells of the embryo adjacent to the original suspensor contribute to it.

The development of the sporophyte was not followed beyond the time of the emergence of the cotyledon and primary root. At the time the lamina of the cotyledon expands only one root, as a rule, could be seen. Some cases were observed (Fig. 5) where a short lateral root had developed



TEXT-FIG. 11. A. Longitudinal section of the stem apex of a young sporophyte. $\times 270$. The large nucleated cell is the apical cell of the stem; L^1 , L^2 , second and third leaves. B. Root apex. x , apical cell. $\times 270$. C. Cross-section of the petiole of the cotyledon. $\times 70$. D. Monarch bundle of primary root. $\times 270$.

from the primary one, but this was probably the result of some injury to the apex of the primary root. The bundle of the first root is usually diarch, as in *B. Lunaria* and *B. virginianum*, and the endodermis is very conspicuous (Text-fig. 10, H). Not infrequently, however, one xylem is suppressed and the bundle is monarch, as in *Ophioglossum vulgatum* (Text-fig. 11, D).

While it is common for more than one archegonium to be fertilized, and several one-celled embryos are frequently found on a gametophyte, in only two cases were two older embryos encountered, and one of these was much better developed than the other, indicating that the smaller one would

finally succumb. Bruchmann found two complete sporophytes attached to the same gametophyte in *B. lunaria*, but the writer found no such cases in the specimens of *B. obliquum* that were examined.

There is no question that the vascular system in the young sporophyte of *B. obliquum* is composed exclusively of leaf- and root-traces. No cauline stele is present, and in this it agrees with *Ophioglossum moluccanum* and the Marattiaceae, especially *Danaea*.¹

The embryos of *B. Lunaria* and *B. virginianum* differ greatly from each other and also from *B. obliquum*. In the former, according to Bruchmann, the early divisions are arranged quadrant-fashion, and the embryo is globular. No definite sequence could be made out in the subsequent divisions, and the embryo retains its globular form, with no trace of the primary organs, which are very late in developing. The root is the first organ to develop, but it is not quite clear from Bruchmann's account as to just when and where the root apex is differentiated. The sporophyte, when it breaks through the prothallial tissue, consists of little more than the root and foot. A little later the stem apex is developed in a cleft between the base of the root and the foot. The stem apex remains very inconspicuous, and the first eight to ten leaves are reduced to mere scales, and it is several years before the first functional leaf appears above ground.

In *B. virginianum* the cotyledon is functional, and even better developed than in *B. obliquum*. A large root and massive foot are present, but there is no suspensor, and the cotyledon and root make a sharp angle with each other, and the root is not endogenous in origin. The conspicuous sheath found in the cotyledon of *B. obliquum* is not found in *B. virginianum*.

It is very evident, then, that *B. obliquum* differs in very essential respects from the two other species that have been studied. Of greatest importance are the suspensor, the endogenous origin of the root, and the strongly bipolar arrangement of cotyledon and root. There is thus a marked resemblance on the one hand to *Ophioglossum moluccanum*, and on the other to the Marattiaceae. The presence of a suspensor is shared with *Helminthostachys*,² which on the whole among the Ophioglossaceae seems to be most nearly related to the Marattiaceae. Of the latter *Danaea* closely resembles *B. obliquum*, both in the presence of a suspensor, and in the general structure of the young sporophyte. The genus *Macroglossum* also possesses a conspicuous suspensor.

SUMMARY AND CONCLUSION.

1. The gametophyte and sexual organs of *Botrychium obliquum* do not differ essentially from those of the other species of *Botrychium*.

¹ Mr. Baas-Becking, who is now studying the development of the young sporophyte, has found that the second root originates exactly as in *Danaea*.

² Lang: loc. cit

2. The embryo differs in several important particulars from both *B. Lunaria* and *B. virginianum*. It resembles the latter in having the cotyledon well developed, but differs in the endogenous origin of the root, in the bipolar arrangement of cotyledon and root, and especially in the presence of a suspensor. The embryo is much more like that of some species of *Ophioglossum* and *Danara* than it is like other species of *Botrychium*.

3. The stem apex grows from a single apical cell, which is much like that of *Ophioglossum vulgatum*. The young cotyledon also has a single apical cell.

4. There is a single primary vascular strand which extends without interruption from the cotyledon into the root. There is no cauline stele, and the primary vascular strand is augmented later by additions from the traces of the second and third leaves.

5. The cotyledon has a ternate lamina with dichotomous venation. The bundle of the petiole is collateral in structure.

6. The root early develops a conspicuous tetrahedral apical cell, and its development is much like that of the later roots. The bundle is usually diarch, but may be monarch.

While the similarities in both gametophyte and adult sporophyte indicate a near relationship between the species of *Botrychium*, the remarkable differences in the embryo and young sporophyte of the three species investigated may warrant a division of the genus into three. *B. Lunaria*, *B. obliquum*, and *B. virginianum* represent three types of adult sporophyte which differ in a number of particulars, viz. form, texture, and venation of the leaf; size of sporangium; position of sporangiophore; character of leaf-base.

Prantl¹ recognizes two subgenera, *Eubotrychium* and *Phyllotrichium*, while Milde² divides into *Eubotrychium* and *Osmundopteris*. The first includes all of the species except *B. virginianum* and *B. matricariaefolium*. Most of the species of *Eubotrychium* belong to the 'Ternatum' group, of which *B. obliquum* is an example. These have a fleshy, ternately compound, sterile leaf-segment, while the stalk of the sporangiophore is inserted close to the base of the petiole.

Should further investigation show that the other species of the Ternatum group agree with *B. obliquum* in the structure of the embryo, there would be ample reason for accepting Lyon's genus *Sceptridium*.

The writer believes that *B. virginianum* differs sufficiently from the other species to warrant raising *Osmundopteris* to generic rank, and restricting the name *Botrychium* to *B. Lunaria* and its near allies.

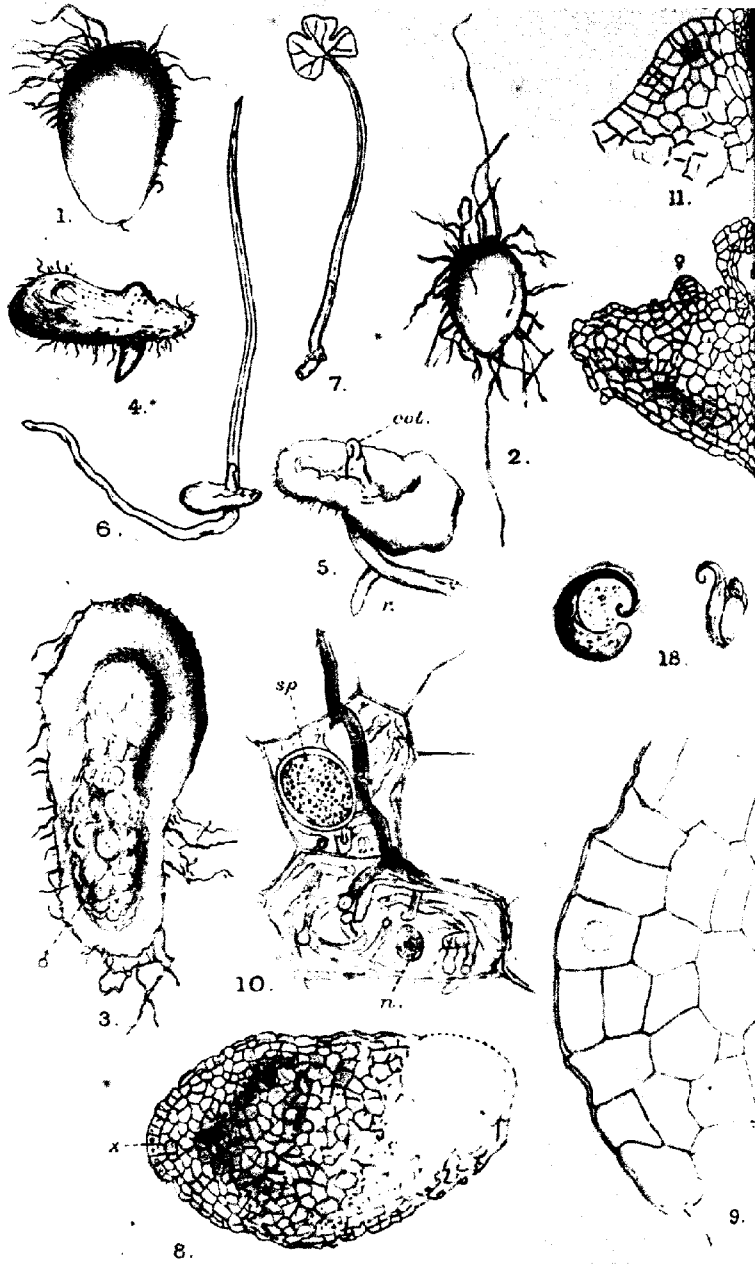
¹ Ber. d. deutsch. bot. Ges., i. 349.

² See Underwood: Our Native Ferns, p. 132. 1888.

EXPLANATION OF PLATE VIII.

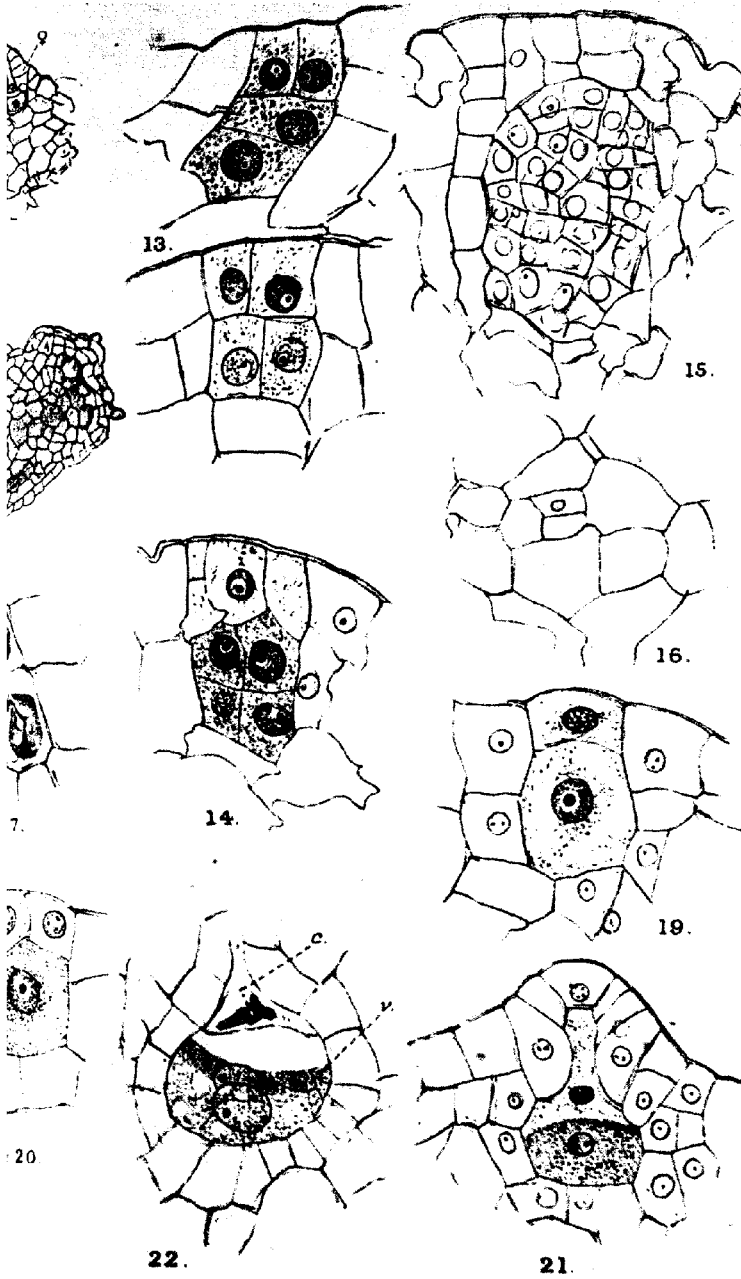
Illustrating Mr. D. H. Campbell's paper on the Gametophyte and Embryo of *Botrychium obliquum*.

- Figs. 1, 2. Young gametophytes seen from above. $\times 20$.
 Fig. 3. An older gametophyte, seen from above, showing the median antheridial ridge.
 Fig. 4. Adult gametophyte, with the root of the young sporophyte emerging on the ventral side. $\times 40$.
 Fig. 5. Gametophyte with young sporophyte attached. The cotyledon emerges from the dorsal surface, the root, *z*, from the ventral side. A lateral root has formed from the primary root. $\times 5$.
 Fig. 6. Gametophyte, with young sporophyte attached. $\times 3$.
 Fig. 7. Young sporophyte, showing the ternate cotyledon. $\times 3$.
 Fig. 8. Median longitudinal section of young gametophyte, showing the growing-point, *z*.
 Fig. 9. The shaded portion indicates the region occupied by the mycorrhiza. $\times 58$.
 Fig. 10. Apical region of the gametophyte. $\times 360$.
 Fig. 11. Two cells from the gametophyte showing the endophytic fungus. $\times 600$. N, nucleus of the gametophytic cell; *sp.*, 'spore' of the fungus.
 Fig. 12. Transverse section through the antheridial ridge of a young gametophyte. $\times 95$. δ , young antheridia; γ , an archegonium.
 Fig. 13. Transverse section of an older gametophyte. $\times 58$. The shaded area indicates the extent of the region occupied by the endophyte.
 Figs. 14, 15. Young antheridia seen in median longitudinal section. $\times 480$.
 Fig. 16. An older stage. $\times 360$. *o*, opercular cell.
 Fig. 17. Surface view of an antheridium, showing the opercular cell, *o*. $\times 360$.
 Fig. 18. Spermatocytes with nearly ripe spermatozooids. $\times 1120$.
 Fig. 19. Two free spermatozooids. $\times 1120$.
 Figs. 20, 21. Very young archegonia. $\times 480$.
 Fig. 22. Archegonium, showing primary neck canal cell. $\times 360$.
 Fig. 23. Venter of a ripe archegonium. The ventral canal cell, *z*, not separated by a wall from the egg-cell. Two nuclei in the neck canal cell.



D.H. Campbell del.

CAMPBELL — BOTRYCHUM.



The Status of the British Rose Forms as determined by their Cytological Behaviour.

BY

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AND

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With Plates IX and X, and five Figures in the Text.

I. INTRODUCTORY.

THE study of the genus *Rosa* was commenced in the year 1913, chiefly with the object of throwing, if possible, some light on one of the most baffling problems presented by the British Flora, that is, the value to be assigned to the various rose forms. This investigation was carried out at first simply from the systematic side, but so hopeless was the task rendered by the extraordinary polymorphism of the plants considered, that quite early it became evident that no real scientific progress could be expected from that standpoint alone. Nevertheless, that very variability provided the clue which we consider destined to establish the true status of the *Rosae*.

Accustomed as we had been to work with hybrids (both plant and animal) of every degree of complexity, we could not help being impressed by the close analogy between the so-called rose species and our artificial hybrid products. The problem, therefore, shifted from the ground of the systematist to that of the geneticist, and we set ourselves to determine, if possible, the hybrid nature, or otherwise, of the plants we were studying. Two lines of approach seemed capable of yielding the desired solution:—

1. Known hybrids like Rosenberg's *Drosophila longifolia* \times *D. rotundifolia* amongst plants, and our hybrid Bistons and Oporabias amongst animals, when examined cytologically, display characteristic anomalies in their maturation divisions; do the *Rosae* exhibit the same behaviour?

2. Recognized plant hybrids generate ill-developed pollen; is that of the *Rosae* equally imperfect?

Although the cytological work was begun the earlier, the second research has been published first (Harrison 1920); still, we must point out that a *résumé* of the cytological results was given in a paper read by one of us at the British Association meeting in August, 1920. Of the latter paper the present account is an expanded statement.

Straightforward as the questions propounded seemed to be at first sight, difficulties were soon encountered, for apomixis occurs in its facultative guise in practically every rose species or microgene. Thus, if the bulk of our roses are hybrids, as the results of both researches indicate, in the *Rosae* as in other genera, *Taraxacum*, *Erigeron*, *Hieracium* and the like, hybridity, polymorphism, and apomixis are related to each other in the way of cause and effect. The elaboration of this point will be reserved for the discussion at the end of the paper.

Material, Methods, &c.

All of the material examined, with the exception of that of *Rosa arvensis* and part of that of *R. rubiginosa*, was collected in Northumberland and Durham and was determined by ourselves. Our determinations we can guarantee to be accurate because the necessary examinations were carried out on the growing bush—a *sine qua non* when dealing with critical microgenes.

In our earlier work the young buds were fixed in Flemming's fluid, but later other fixatives were tested, to be abandoned finally in favour of Carnoy's fluid, which, for the end in view, proved the best reagent.

The sections were cut to a thickness of 5μ , stained with Heidenhain's iron-alum-haematoxylin, and mounted in balsam without a counter-stain.

Very early indeed we became aware that in the genus microspore formation occurred of two types, one perfectly normal and the other exhibiting irregularities, varying with the microgene under investigation, but nevertheless always of the same order. Furthermore, it appeared that the abnormalities occurred in forms with high chromosome numbers, but were almost absent in those with lower complements, i. e. in microgenes with a haploid number of 7 or 14. Incidentally, it will be evident that Strasburger was in error in fixing the fundamental chromosome number in *Rosa* as 8, the correct figure being 7.

The most frequent form examined with this latter haploid number was one of the *Systylae*, *Rosa arvensis*, and upon that species, aided by reference to *R. pimpinellifolia*, we shall found the description of events in the meiotic phase of a normal rose to be utilized subsequently in comparisons with apomalous forms.

II. THE MEIOTIC PHASE IN THE SPECIES BEHAVING NORMALLY.

(a) *Rosa arvensis*, Huds.

As this is not intended to be a critical survey of the cytology of *Rosa*, but is rather an inquiry into the status of its British members, the series of sections figured here simply displays representative stages in pollen-formation, sufficiently complete in themselves to show that in the truly sexual roses the general course of events differ not at all from that followed in typical phanerogams.

The stages chosen for figuring commence with Pl. IX, Fig. 1, in which we depict the resting nucleus just preparing for synapsis by an increase in the bulk of its chromatin. Onward from this there is a progressive massing of the chromatin to one side of the nucleus, where it is arranged, more or less neatly, between the nucleolus and the wall. When synapsis is at its maximum intensity, the chromatic clump becomes exceedingly dense and granular, as is shown in Pl. IX, Fig. 2. As the synaptic knot unravels, obviously looped sections are thrown out into the nucleus (Pl. IX, Fig. 3). Next the loops extend until the whole nuclear cavity is filled, and the ordinary hollow spireme stage is reached. The beaded spireme then thickens, and, as it does so, reveals itself as made up of seven long loops. The succeeding stage shows the units of the bivalents twisted round each other (Pl. IX, Fig. 7). Henceforth the chromosomes concentrate and thicken with an accompanying increase of their staining powers and a diminution in the case of the nucleolus to more or less cytoplasmic values (Pl. IX, Fig. 1c). Subsequent to this an irregular multipolar spindle (Pl. IX, Fig. 11) of a transient nature arises, passing by imperceptible steps into the typical bipolar form. At this point the somewhat oval chromosomes reach the spindle (Pl. IX Figs. 12 and 13) when, as is quite plain, they lie with perfect regularity on the equatorial plate; now, too, the nucleolus has disappeared. The separation of the bivalents takes place with the utmost regularity, and the chromosomes, without exception, reach the poles simultaneously (Pl. IX, Fig. 14).

No hint is given here of hybridity; the perfect assembling of the chromosomes on the equatorial plate, their synchronizing in doing so, the neat arrangement of the separated chromosomes on the spindle, as they pass to the poles, force upon one's mind the certainty that in this species at least, no hybridity exist either patent or latent. There is absolutely no suggestion of lack of pairing, irregularity in passing to and from the equatorial plate, so obvious in the heterotype division of *Drosophila obscura*, or of the first maturation division of our *Lyctia-Nyctia* and *Operabia* moth hybrids.

In Pl. IX, Fig. 15 the interkinesis between the heterotype and the homotype divisions may be seen with the chromosomes represented so that no longer can the chromosome number be made out. At this stage the nucleolus comes once more into view.

The homotype divisions succeeding this call for no special remark, so great are their uniformity and fidelity to type. Perhaps we should state that the two spindles, for the most part, as shown in Pl. IX, Fig. 18, lie at right angles to one another.

As the chromosomes reach the poles they are grouped together, but immediately separate, becoming granular and once again uncountable in the process (Pl. IX, Fig. 19). Once more, too, the nucleolus becomes an evident adjunct of the nucleus. Finally, and with but little delay, the mother-cell divides into four spores, which with the secretion of a cell wall bring into being a perfectly ordinary tetrad and, later, functional pollen (Pl. IX, Fig. 20).

Ordinarily a brief account of the course of events in the meiotic phase of a plant, such as we set out to give, would be complete at this point. However, in view of the extraordinary discrepancy between the somatic and semi-reduced chromosome number in the majority of roses on the one hand, and of the regular haploid and diploid relationship in those of *Rosa arvensis* and the average phanerogam on the other, the facts must be emphasized by figures of a somatic mitosis in that rose.

Pl. IX, Fig. 21 shows a somatic nucleus in late prophase, exhibiting very clearly the splitting in preparation for the ensuing division, whilst Fig. 22 shows a typical equatorial plate. In both, without the faintest possibility of equivocation, the somatic number is 14. In other words, the haploid number for *Rosa arvensis* is 7 and its diploid 14, these numbers bearing the normal relation to each other.

(b) *Rosa rugosa*, Thun.

Rosa rugosa, an occasional escape or covert shrub in this district, displays essentially similar features to those described for *R. arvensis*, its diploid number likewise being 14 and its haploid 7. Only one fact seems worthy of special mention in connexion with this rose, and that is the very considerable quantity of granular chromatin matter present in the nucleus during diakinesis. Otherwise it is very ordinary in its behaviour. Under these circumstances, to avoid unnecessary duplication, we do not supply a series of figures for it, but content ourselves by inserting a good example of a homotype anaphase (Pl. IX, Fig. 17) to illustrate its perfect regularity at a stage when most rose forms exhibit their worst behaviour.

(c) *Rosa pimpinellifolia*, L.

With this form we include the hispid glandular peduncled *R. spinosissima* as well as a tall sterile bush indistinguishable otherwise than in its stature and sterility from *R. pimpinellifolia*. All three forms, in contrast to the irregular tetraploid *Villosae*, appear, save in one slight detail in the sterile type, as wholly normal tetraploids.

Cytologically speaking, they deviate but slightly from the path followed in *Rosa arvensis*, but to emphasize their great approximation in this respect and their total disagreement from all other British roses some account must be given.

As in *R. arvensis*, the granular chromatin of the resting nucleus of the pollen mother-cell masses itself into a dense synaptic knot, which persists as such for some fairly lengthy period whilst the mother cells are separating. In the end loops are thrown outward to the periphery of the nucleus; after this the knot gradually unloosens to build up the apparently continuous spireme (Pl. IX, Fig. 4). Presently this thread thickens and shortens to be seen as being made up of loops divided lengthwise either completely or sectionally, thereby evolving the twisted hairpins of Pl. IX, Figs. 5 and 6. To the interpretation of this split we shall revert under *Rosa Sabini*. These bivalent loops, the halves of which at first are so attenuated as to be barely visible, rapidly condense to yield the V's, X's, and O's of a typical diakinesis (Figs. 8 and 9).

The 14 bivalent chromosomes then approach one another with the accompanying development of a multipolar spindle. This, however, speedily gives place to the bipolar variety, and the chromosomes take their places on the equatorial plate. This event occurs in the usual fashion in fertile *pimpinellifolia* and in the *spinosissima* segregate; on the contrary in the sterile plant¹ one chromosome may be detected as a fifteenth one lying in the region of one of the poles (Text-fig. 1). In all instances, except for the larger chromosome number, the further stages are exactly parallel to those of *Rosa arvensis* and *R. rugosa*.



TEXT-FIG. 1. *R. pimpinellifolia*. Heterotype metaphase showing odd chromosome like body. $\times 3,000$.

The somatic count may be made out to be 28 in the trio of forms, although some small amount of difficulty may arise in the process, owing to the length and curvature of the chromosomes (Pl. IX, Fig. 23). That the haploid number is 14, Fig. 22 amply demonstrates.

III. THE CYTOLOGY OF THE PHENHYBRIDS OF *ROSA*.

(a) *Rosa Sabini* (= *R. pimpinellifolia* \times *R. tomentosa* var. *sylvestris*).

The plant about to be considered belongs to that heterogeneous assemblage known to the early rhodologists as *Rosa involuta*, Smith. Now, of course, it is universally deemed to be a hybrid between *Rosa pimpinelli-*

¹ These tall plants are always sterile in Durham, and this slight abnormality; their tallness and sterility, may all be relics of some crossing in the previous history of the form.

folia and a member of the *Villosae*, using the sectional name in its widest sense as covering the *Rosa mollis*, *R. omissa*, *R. tomentosa*, and *R. foetida* groups.

After careful study in the field of the bush whence our preparations were derived, and making due allowance for its neighbours, we have decided that its exact parentage is R. pimpinellifolia x R. sylvestris—seemingly quite a frequent combination in our two counties; as confirmatory proof of its hybrid nature, if any were needed, we may say that, wherever we have come into contact with it, it has been uniformly barren—a point neglected by most previous writers, who have merely observed its wealth of blossom in June and July.

Recognizing the importance of determining the somatic chromosome number correctly in order to procure confirmation of our diagnosis of the parentage, careful counts were made from clear prophase in the styles where, as is general in the genus, such figures are very frequent. Such counts prove difficult to make accurately, owing to the unusually large number of chromosomes present and their elongated shape. Nevertheless, in favourable nuclei, the number has been ascertained with complete exactitude to be 42 (Text-fig. 2, a). The precise significance of this number will be considered later.

Coming now to the behaviour of the chromosomes during meiosis, unlike what we have noted in moth hybrids of the genus *Lycia*, the stages leading from the reticulum onward through synapsis seem absolutely normal. Pl. X, Fig. 26, showing the synapctic knot at its greatest contraction, differs in no wise from the same stage depicted for *Rosa arvensis* on Pl. IX, Fig. 2, and is quite representative of the number examined. By degrees the open spireme stage is attained in which the thread, in spite of its delicacy, can be followed in its continuity with some amount of ease as it winds around the periphery and elsewhere in the nucleus (see Pl. X, Fig. 27). With the passing of this stage, certain of its threads are seen to lie parallel, thus initiating the formation of the bivalents. Here we must remark that it would appear a fair deduction, when one allows for the future development of bivalents and univalents *pari passu*, to assert that the chromatic thread, as it emerges from synapsis is still univalent; one cannot conceive of its being univalent and bivalent at one and the same time. This should be set against the parasyndetic appearance of the stage of *R. pimpinellifolia* depicted on Pl. IX, Figs. 5-7, as it seems unlikely that the species should differ in this respect.

Gradually the chromosomes thicken and concentrate, and very early indeed it becomes evident that whilst certain individuals are, without any possible doubt, bivalent, others just as assuredly remain unpaired and can often enough be detected as marvellously straight single rods. Concentration proceeds regularly in bivalent and univalent alike until in

diakinesis (figured on Pl. X, Fig. 29) both types are determinable with the greatest possible ease.

Compared with similar stages in other plants, subsequent events follow an anomalous course, which, however, in the light of extensive studies in *Rosa*, is found to be more or less characteristic of the genus. In assembling on the equatorial plate for the heterotype division, the 14 bivalents mass themselves round the central point of the spindle with the utmost regularity. Later the univalents follow with less exactness and arrange themselves in a complete ring round the paired individuals (Text-fig. 2, *b*). In good sections showing horizontal plates, the total number of chromosomes is 28, which agrees perfectly with the somatic count of 42, since 14 bivalents + 14 univalents yield a total of 42.

The anaphase, too, is noteworthy, for it occurs in two distinct phases, one involving the bivalents and the later one the univalents. Often enough the former is almost over (Pl. X, Fig. 33), and even sometimes merging into the telophase, before the univalents split and attempt to move. It would almost appear that the force urging the chromosomes had spent itself with the passage of the bivalents, since the split halves of the remainder lag, wander, and occasionally get lost. However, in the majority of cases, the greater portion of the split halves unite in the telophase with the 14 whole chromosomes already awaiting them, to constitute one daughter nucleus. Still a few of the laggards mass themselves apart into groups of varying size, and finally develop into micronuclei (see Pl. X, Fig. 34). An interesting feature in connexion with the heterotype spindle, in this form, is its close proximity to the periphery of the mother-cell, as is well shown on Pl. X, Fig. 34.

Very soon after the short interkinetic period preparation is made for the homotype division. The fact that many of the split halves of the univalents are included in the two major nuclei becomes very apparent here, for counts on good homotype plates (Pl. X, Fig. 38), differing widely in number it is true, but often reaching the twenties, can be made. Once again events move abnormally. As before, the chromosomes descended from the original 14 bivalents travel first to the centre of the plate followed by a varying number of halves. The former divide precociously and pass to the telophase with such rapidity that before many of the splitting halves can approach them the daughter nuclei may be reconstructed. Thus there is a general tendency for the major nuclei, representing the genuine nuclei of the tetrad, to be built up from 14 chromosomes. Curiously enough, the dividing halves derived from the univalents seen in the original diakinesis may also show a more or less pronounced inclination to keep together, although they may diverge. When they do act as a unit, the 'tetrad' contains eight major nuclei and may therefore more appropriately be termed an 'octad'. On the other hand, if they separate, when

due allowance is made for the micronuclei developed after the heterotype division, large numbers may be present.

However, whether the number of microspores is great or small, their fate is already sealed, for they all collapse.

In view of the great theoretical importance attached to the fact an occurrence observed during the hybrid homotype division must be mentioned. In one pollen mother-cell, instead of the usual pair one giant spindle had been formed involving the whole of the split univalents and the bivalents. Thus in the late anaphase the huge array of chromosomes could be seen lying in two orderly groups, just as if the cell were pursuing a normal mitosis (see Pl. X, Fig. 4c). Had development been allowed to proceed, resulting in a functional pollen grain, we should have had a gamete possessing all the necessary qualifications for producing a new plant, orthoploid in its chromosome number, but with a complement much higher than those of the plants from which it was generated. Further reference will be made to this phenomenon later.

Finally, on account of its extreme importance, we must insist that the meiotic plan just traced is that displayed by a plant admitted by every rose student in England to be of hybrid origin.

(b) *Rosa hybrid pimpinellifolia* × (*pimpinellifolia* × *coriifolia*).

Of the hybrid nature of this form no doubt can be entertained, and on grounds detailed at length in the concluding remarks we have determined that its parents are as stated above.

As with the plant just considered, to fix its parentage and to enable us to comprehend fully the nature of the chromosomes to be accounted for in the meiotic stages, careful counts of the number of chromosomes occurring in the nuclei of the cells of the style were made. The numbers so obtained seemed to vary slightly even when the accuracy seemed guaranteed, so that whilst in most cells 28 chromosomes were to be made out (Text-fig. 2, c), in others 29 appeared to be present.

When seen in polar view the plates of the heterotype division (Text-fig. 2, d) gave without exception counts of 14, indicating that, in all probability, there were on the plate, as in the type previously studied, 14 pairs of bivalents. However, views of the spindle in profile during the anaphase demonstrated that widely separated chromatin fragments, and even possibly the halves of split univalents (Pl. X, Fig. 35), lay along the spindle fibres.

Thus the interkinesis before the homotype divisions held in store few untypical features likely to end in abnormalities during the division, thereby ensuring that reasonable numbers of perfect microspores should result. This was reflected for the most part in the appearance of a regular tetrad of four nuclei. Rarely, however, five or six could be observed. Moreover, too, the condition of the pollen varied with the loculus, some loculi containing

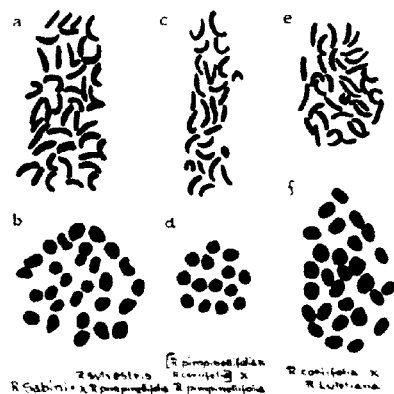
up to 100 per cent. of good grains, whereas in others the percentage was much lower. Frequently the pollen did not mature at all.

Making the requisite allowance for the much smaller chromosome complement, the whole of the stages examined showed no significant deviation from what obtained in the preceding type.

(c) *Rosa coriifolia* var. *Lintoni* \times *R. lutetiana*.

For reasons put forward in the concluding remarks (p. 177) we have decided that the parentage of the plant about to come under consideration is what we have just stated, and a further critical study of its neighbours made recently has sufficed to confirm our opinion.

Buds were collected from the plant at exactly the same time as we made our gatherings of *Rosa omis*, *R. rubiginosa* var. *apricorum*, *R. coriifolia* var. *Lintoni*, *R. dumetorum* var. *hemitricha*, and *R. lutetiana*. After fixing the buds of the whole lot for the same length of time in portions of the same stock of Carnoy's fluid they were cut, stained, and mounted. For some unknown reason, whilst the first four took the stain readily, we had the utmost difficulty in overcoming the capricious behaviour of this rose and *R. lutetiana* towards iron-haematoxylin. Even at its best it never took this stain like the rest of the roses we have mounted.



TEXT-FIG. 2. Somatic and heterotype metaphases in hybrids. $\times 3,000$.

Basing our opinion upon a number of prophases of which Text-fig. 2, c, is quite typical, we have no hesitation in fixing its somatic chromosome number at 35, and we are convinced that seven of these were obtained from the *lutetiana* parent and twenty-eight from the other. There would thus be brought in from the latter twenty-one, which are presumably unaccustomed to act as homologues of others in the stages leading to the semitypical reduction division, and seven that were descended from chromosomes capable of doing so. From *Rosa lutetiana* came in seven of like character to the latter group. Naturally one would expect that the last two sets would be homologous to one another and be paired in the imperfect reduction division.

In Text-fig. 2, f, the seven bivalents may readily be discerned lying in

a horseshoe shape with some of the univalents included within its arms, although most are arranged so as to surround them partially. Both at earlier stages and subsequently matters move in the very strange fashion described at some length for *R. coriifolia* and, save that the bivalents in this case are seven in number, for *R. Sabini*.

In the interkinesis we see two major nuclei, with varying numbers of micronuclei as well as with odd degenerating chromosomes. In the homotype the movement of the chromosomes to the spindle is even more disturbed by the hybrid combination at work, and despite the fact that the scheme followed is that typical of pentaploid forms, the tetrad is so very abnormal that almost the whole of the microspores are abortive. Two per cent. of good grains was the best count made from a microscopical inspection of the contents of a number of anthers.

Similarly, megaspore formation cannot have been uniformly successful, for, although the bush produces some well-developed seeds by a process of apomixis, only a small proportion of the fruits swell satisfactorily as the seeds ripen within; the remainder simply contain a mass of chaffy scales.

IV. THE CYTOLOGY OF THE PENTAPLOID GROUP.

(a) *The Afzelianae.*

The section *Afzelianae* comprises all of the segregates classified by English botanists, as genuine species, under the old collective names *Rosa coriifolia* and *R. glauca*. However, so closely do the forms assigned to one or other of these supposed cardinal species approach one another, that no difficulty is encountered in finding two bushes absolutely identical, save for some microscopic pilosity on the midribs of the leaflets of one, which suffices to drive that form into *R. coriifolia* and the other into *R. glauca*. From this the recognition of the two seems ridiculous, and to avoid their unnatural separation Almqvist united them under the oldest collective name capable of covering both sets of plants; that name was *Rosa Afzeliana*. At the same time, he removes the glandular microgenes, considered by most workers on the genus *Rosa* to be true segregates of *R. coriifolia*, to the section *Rubiginosae*. In this, whilst we recognize some sound reasons for the procedure, we are unable to follow him; the gradual transition from *R. coriifolia* (type) to *R. Lintoni* renders it almost impossible to differentiate virescent forms near the type from the less glandular varieties of the roses so transferred. Thus, our conception of the *Afzelianae* differs from what Almqvist includes under the name, inasmuch as, in our opinion, the section contains the whole of the old 'species' *R. coriifolia* and *R. glauca*.

(1) *R. coriifolia*, Fries.

This plant was almost the first to which we turned our attention. We were very fortunate in our choice. In it we secured almost ideal material

for disclosing the peculiarities characterizing the meiotic phase in *Rosa*. Examination of its somatic mitosis very soon brought us into contact with the unorthodox chromosome number of thirty-five. Although influenced, no doubt, by the apparent certainty of Strasburger's count of thirty-two in other roses, and likewise by the improbability of the occurrence of pentaploid species, we were inclined to think that our counts were rendered unduly high by the presence of precociously dividing chromosomes, by their being cut accidentally by the microtome or split by other means. However, the somatic number is undoubtedly thirty-five, and Text-fig. 3, *a*, adequately represents the large number of somatic prophase from which we have made counts.

In the meiotic stages, when direct comparisons are made with *R. arvensis* and *R. pimpinellifolia* at parallel points, whether preceding, during, or just subsequent to synapsis, no discrepancies are to be noted. There is the same gradual passage from the reticulum to close synapsis yielding in its turn to an equally perfect hollow spireme. Notwithstanding this close resemblance here, in the later stages they disagree in almost every detail, and to determine the exact import of the striking differences manifested the outline of events during meiosis described for *R. Sabini* must be carefully borne in mind. Coupled with this reference to *R. Sabini* must be the fact that this rose is admitted to be of hybrid origin by every one competent to put forward an opinion.

Returning now to *R. coriifolia*, after the formation of the loops from the spireme in the heterotype prophase, we have, as with *R. arvensis*, a thickening and condensation of the chromosomes, which, however, are seen to be behaving dissimilarly. Some are clearly discernible as threads folding and interlacing on themselves, as the sides of the individual loops close up; others are just as certainly isolated portions, severed, so to speak, from the contracting spireme. In this fashion, we glean that the plan followed adheres rather to that of *R. Sabini* than to that of *R. arvensis*. Further, the view is strengthened in diakinesis, for the chromosomes in sight are very obviously partly bivalents and partly univalents. Nevertheless, although the bivalent combinations are decidedly in the minority, no perfect count can be made of the precise number of each type present, for the total number of chromosomes is too great.

As the chromosomes pass to the spindle all this uncertainty passes away. In a regular manner, observed in one heterotype metaphase after another, the bivalents arrange themselves with mathematical precision immediately around the centre of the equatorial plate (see Pl. X, Fig. 3c), and in doing so reveal themselves as being seven in number (see Text-fig. 3, *f*). Next, the univalents approach and encircle them, in general with considerable regularity, giving beautifully clear plates, on which twenty-eight chromosomes lie visible, the seven inner bivalents standing out as larger

and blacker than the remainder. First the bivalents separate in their anaphase, actually reaching the poles ere their companions divide (see Pl. X, Fig. 36).

When the latter do so we have an anaphase totally distinct and much less in accord with type. With extraordinary fidelity the events recorded for *R. Sabini* are reproduced. Nor does the resemblance end here; just as related for that plant, the attraction (or repulsion?) urging the chromosomes to the poles seems to lose its strength long before the whole of the split univalents reach their goal. Some of the failures wander and degenerate, whilst others join company in little groups of three and four, to appear later in interkinesis, as micronuclei, comparable with those recorded long ago for the same stage in *Hemerocallis fulva* by Strasburger and Juel. That the majority do manage to reach the seven separated bivalents in the 'polar cap' is demonstrated in a convincing fashion in the homotype division, for there, once again, the plates, ideal in their simplicity, show numbers varying from sixteen to twenty-five. Once more the seven whole chromosomes, derived from the former associated pairs, travel to the plate first and arrange themselves centrally, with somewhat less precision, to be enclosed later by the chromosomes representing the split halves of the heterotype phase. An equatorial division then takes place for the first set, when their halves travel rapidly towards the poles (see Pl. X, Fig. 39). Although delayed in the end the others divide, but rarely, if ever, pass more than two-thirds of the way up the spindle fibres. As the outcome of this premature halting the major daughter nuclei contain almost uniformly seven chromosomes, that number having been determined in scores of cases. Nor is there any great difficulty in making out the same number, when the chromosomes display themselves with much less sharpness of outline, in the major nuclei of the final octad.

To go back to the fate of the delayed number; with some regularity, much greater than in the analogous case of *R. Sabini*, they tend to crowd together, thus entailing, with the reconstruction of the daughter nuclei, the development of eight major groups. From this we learn that usually, as already stated, an octad rather than a tetrad is generated; still, however, we must bear in mind that the term, although allowable in the circumstances, is not strictly correct, when due cognizance is taken of the number of micronuclei likewise included (see Pl. X, Fig. 41). Be that as it may we have, arising from the pollen mother-cell and lying somewhat carefully spaced within the octad, eight nuclei, markedly different from the rest. These, acting independently, take unto themselves separate portions of cytoplasm, secrete the normal exine, and finally yield pollen grains. The fate of the micronuclei varies. If entrapped within the sphere of influence of one of their larger comrades they are included within the microspore to which it gives rise. On the other hand, one, two, three, or even more

acting together may take part of the cytoplasm to themselves, to develop in the end into pollen grains of dimensions varying with the chromatin content of the nuclei, whence they originate.

After this, in the bulk of the pollen grains, degeneration sets in, although a fair proportion, as proved by direct observation, are quite functional.

Giving due weight to the exceedingly close resemblance between the whole of the events during microspore formation in *R. coriifolia* and *R. Sabini*, from the typical preparatory stages to the anomalous later ones, and recollecting the perfectly ordinary and orderly meiotic phase in *R. arvensis*, we are forced to the opinion that the same agency, hybridity, is responsible in the first-named pair of plants for their common peculiarities. Undoubtedly, the very least one can say is that the cytological behaviour of *R. coriifolia* is that of a hybrid.

(2) *Rosa coriifolia* var. *Lintoni*, Scheutz.

This rose is one of the glandular members of the Afzelianae and would, by Almqvist, be regarded as appertaining to the Rubiginosae. We prefer, for reasons perfectly apparent when the rose is studied as it grows, to leave the rose as placed by English rose students.

Its somatic chromosome number, as in the case of the type, adds up to thirty-five. Further, as far as the chromosomes are concerned, we cannot perceive any divergence of behaviour between the two. As in *Rosa coriifolia*, Fr. they appear in the heterotype division as seven bivalents and twenty-one univalents, and the descendants of these are distributed in the final microspores in the same general fashion.

One important point of difference is manifested; without exception, during synapsis and the stages intervening between that and diakinesis, the nucleolus appears to be doubled. Often enough, there are actually two nucleoli present; less frequently they assume an exact dumb-bell shape, whilst most commonly we have the appearance of a minor nucleolus attached to a greater. Whether this is to be interpreted as a vigorous budding of the nucleolus during the most critical period of the existence of the nucleus we know not, but we are not inclined to admit that explanation.

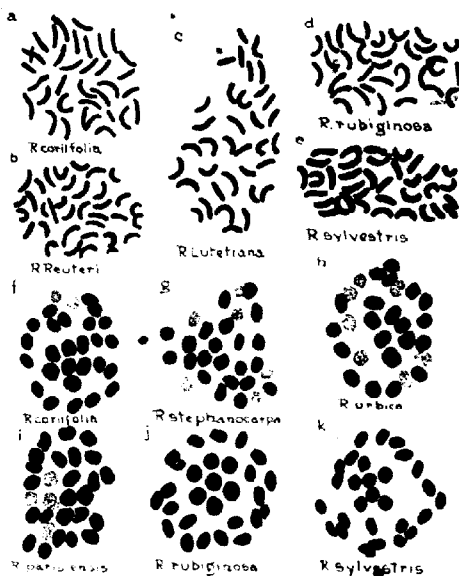
In any case, the exact significance of the phenomenon, and why the two allied forms should so consistently differ in this respect, we cannot state.

(3) *The Rosa glauca group.*

Rosa glauca will not delay us long. Just as in the other groups more than one microgene was examined, and these were *Rosa Kenturi*, God. (uniserrate), *R. venosa*, Schwartz, *R. subcristata*, Baker (biserrate), and *R. stephanocarpa*, Dés. and Rip. (biserrate and with subfoliar glands).

Once more we discovered that we were dealing with members of the pentaploid group with a somatic chromosome complement of thirty-five, as Text-fig. 3, *b*, from *R. Reuteri* will suffice to show.

Except for one single point, that we fancied the synaptic knot to be tighter than in the allied *R. coriifolia*, the cytology of the two Afzeliana types, completely coincides. Text-fig. 3, *g*, from *R. stephanocarpa* will prove that this is the case as far as the arrangement of the chromosomes is concerned. There the seven bivalents can be made out surrounded by



TEXT-FIG. 3. The pentaploid roses. Somatic and heterotype equatorial plates. $\times 3,000$.

the complementary univalents. Giving due weight to their agreement, no additional treatment seems necessary.

(b) *The Eucaninae.*

To us the term 'Eucaninae' covers all the 'ultra'-microspecies ranged in Wolley-Dod's list under the *canina* and *dumetorum* groups; it thus appears as an aggregate systematically, if not genetically, equivalent to a collective species.

We regard *R. dumetorum*, once deemed a very important macrospecies, as quite an unimportant entity when placed alongside its associates.

Notwithstanding this, to indicate precisely what forms we have studied, we give their names as set down in the list to which we have

just referred, and likewise keep the hairy-leaved forms separated from their glabrous allies.

(1) *Rosa canina*, Linn.

The segregates brought under examination were *R. flexibilis*, Déség., *R. fallens*, Déség., *R. separabilis*, Déség., and *R. parisiensis*, Rouy, the first three forms being uniserrate and the other biserrate.

Without exception all possessed a chromosome count of thirty-five in the nuclei of the somatic cells (Text-fig. 3, c, from *R. luteana*) and were therefore pentaploid.

As with *R. coriifolia*, these chromosomes were of two types, as revealed during meiosis, fourteen being bivalents and twenty-one univalents (see Pl. X, Fig. 28). Text-fig. 3, i, represents a heterotype plate from the pollen mother-cell of *R. parisiensis*, and, as in *R. coriifolia*, the bivalents are arranged centrally with the univalents round them. Whilst generally the phenomena displayed during the stages immediately preceding the heterotype division and the distribution of the chromosomes during the division itself very closely resemble that visible in *R. coriifolia* (and therefore not requiring a full description), there is a more emphatic raggedness on both heterotype and homotype spindles, with regard to the univalents. This involves a greater abundance of micronuclei in the interkinesis between the maturation divisions, and a consequent accentuation of this number in the subsequent 'octad'.

However, as the seven chromosomes of the 'cap' seem never to be affected, this would appear, in some way, to be an advantage, for the functional pollen grains are bound to be much more generally endowed with that chromosome combination. In these plants, up to 50 per cent. of good pollen in the anther is not an unusual occurrence (see Pl. X, Fig. 44). This, in its turn, explains why, compared with the Afzelianae, the Eucaninae are more often fertilised and less prone therefore to apomixis.

(2) *Rosa dumetorum*, Thuill.

Rosa urbica, Lem., a uniserrate form, and *R. hemitricha*, Rip., a biserrate rose, yielded our material. Both microgenes are pentaploid. Once more the complement of thirty-five comprises fourteen destined to play the part of bivalents in the reduction division and twenty-one that of univalents, as the heterotype plate on Text-fig. 3, h, will show for *R. urbica*.

In every detail the description of events given for the *canina* and *coriifolia* segregates, with the qualifications mentioned under the former, apply here; to repeat them would thus be superfluous. Still, one fact should be noted; in the *hemitricha* form a much greater tendency seems to be manifested for the micronuclei to collaborate with their bigger companions in the development of the tetrad, so that multinucleate microspores are not uncommon. Also, necessarily, the concomitant of this

is aborted pollen and a lack of intensity of the orange colour, when contrasted with that of pollen of a superior grade, seen in the other *canina* and *dumetorum* segregates. At its very best *R. hemitricha* has only produced 15 per cent. of perfect pollen in its anthers.

As in the Afzelianae, so in the Eucaniae, we regard the abnormalities in cytology as pointing directly to hybridity.

(c) *The Rubiginosae.*

Curiously enough, considered in conjunction with our matured conclusions that nearly the whole of the roses are hybrids, the statement of Boitard in 1836 that many well-known rose growers had obtained seedlings of *Rosa ferox* from *R. rubiginosa* seems very illuminating. However, our experiments showed emphatically that *R. rubiginosa* var. *comosa*, Rip., was facultatively and generally apomictical, so that seedlings from the same plant always agreed amongst themselves and with their parent in characteristics.

This section is not well represented in this district, so that only two microgenes, *R. comosa* and *R. apricorum*, Rip., were studied, our local material of the forms being supplemented by some from Bedfordshire.

In harmony with the Afzelianae and Eucaniae, the Rubiginosae proved members of the pentaploid series, having a somatic chromosome number of thirty-five reducing to twenty-eight for the heterotype division (see Text-fig. 3, *d* and *j*). That they mass themselves on the equatorial plate as in the other sections will easily be perceived from the same figure, and also from that on Pl. X, Fig. 31, depicting a heterotype metaphase in profile.

Of the forms previously under review the Rubiginosae most nearly approach *R. hemitricha*, so small is the proportion of pollen capable of germination developed. If the conditions in that plant be borne in mind, coupled with what prevailed in *R. coriifolia*, a good notion will be gained of the meiotic phase in the Rubiginosae; thus unprofitable repetition will be avoided (see also Pl. X, Fig. 42).

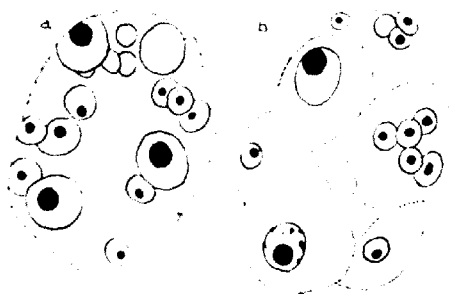
One occurrence must be singled out for special reference. In animal hybrids tripolar spindles in the maturation divisions are quite common, whilst in plants, on the contrary, although a tendency to multipolar spindles is the rule, they always yield to the bipolar type; in *R. apricorum* tripolar spindles occur freely, and, what is more, persist, thus forming one of the factors in the wholesale failure of pollen in these microgenes (see Pl. X, Fig. 32).

This, again, is one of the many features driving home and emphasizing the hybrid origin of practically every British rose.

(d) *The Tomentosae.*

Since *Rosa Sherardi*, Dav., has been transferred to the Villosae, the section Tomentosae, as studied by us, is represented by two forms, *R. sylvestris*, Woods, and *R. scabriuscula*, Sm.

In their somatic chromosome numbers both are in perfect agreement with the Afzelianae and Eucaninae, and are thus pentaploid. The meiotic features, particularly in *R. sylvestris*, are more atypical than in any form as yet described. The early stages up to diakinesis agree likewise with the pentaploids, but on the initiation of the heterotype spindle marked divergences become apparent. Owing to this increased disturbance, or rather as one of the causes thereof, the difference in behaviour between the bivalents and univalents is much less noticeable. Indeed, it often appears as if many of the chromosomes never reach the equator of the spindle at all, but begin to divide wherever they encounter a spindle fibre. The type of spindle shown more nearly approaches that of *Rosa Sabini* at the same stage, although, as in that rose, an excellent polar view of the metaphase is occasionally obtained (Text-fig. 3, *k*). However, the untidy appearance of the assembling chromosomes in *R. Sabini* depends almost certainly on the



TEXT-FIG. 4. *R. sylvestris*. 'Octad' before and after division. $\times 3,000$.

difference in size and shape of the chromosomes contributed by the parents, and, moreover, the usual double anaphase ensues in its entirety. On the other hand, there is no period of demarcation between the successive anaphases in the heterotype division of *R. sylvestris*. The chromosomes behave very curiously and seem to lose their shapes and are pulled out into strings and irregular masses. Notwithstanding this, it is in every way probable that all of the bivalent daughter chromosomes, accompanied by a few of the others, reach the poles. The laggards become surrounded by nuclear walls, forming karyomere-like structures, even before the disappearance of the spindle. Hence, in interkinesis, two larger nuclei, as well as a great number of smaller ones, are visible.

The homotype division is so irregular that it is difficult to distinguish the positions of the main spindles. Almost certainly minor spindles interfere with the major ones. As a consequence, what ought to have been the tetrad contains numerous nuclei of varying size. Text-fig. 4, *a*, is not at

all an extreme example of this, and *b* shows the division into the usual octad in which almost all of the pollen grains are multinucleate (see also Pl. X, Fig. 43).

This accentuated irregularity would seem to suggest that some of the *tomentosa* forms may be very recent, nay even F_1 crosses. Pl. X, Fig. 45 illustrates the regularity of the somatic mitosis even here.

V. THE CYTOLOGY OF THE TETRAPLOID TYPES.

The Villosae.

This type, as far as the British roses go, comprises within its limits nothing but *Villosa* segregates, and amongst those prepared for study were representatives of the *Rosa mollis* group (*R. mollis*, Sm., and *R. coerulescens*, Woods) as well as of the *R. omissa*¹ group (*R. omissa*, Déség., *R. suberecta*, Ley., and *R. Sherardi*, Dav.).

In the case of four of these no further indications of the plant intended are necessary, but, in view of Täckholm's² pronouncement as to the unconvertibility of *R. omissa* to the tetraploid type, and our proof that it is tetraploid, the exact plant we studied must be defined with complete accuracy.

Probably no British worker has a better comprehension of *R. omissa* than the veteran W. Barclay, and direct comparisons made between his gatherings and Swiss type-forms from Déséglise (the describer of the species) have demonstrated beyond cavil that they must be regarded as conspecific. We possessed specimens of Barclay's collecting and naming, and with these the bushes whence we derived our material are practically identical. Further, we are in a position to confirm the accuracy of our determination indirectly. As Almqvist, the well-known Swedish rhodologist who named Täckholm's plants, needed British roses for examination in order to fix the range of the forms of his new system (and not because we doubted our own namings), we sent him a score of Northumberland and Durham examples with three of Barclay's; all, without exception, were returned as *Rosa mollitrichophylla*, Almq. This confirms our belief that Barclay and ourselves were dealing with the same plant and also, as we already knew, that our *omissa* was a *Villosa* rather than the *Tomentosa* form it had been assumed to be. What Almqvist and therefore Täckholm understand by *Rosa omissa* will appear as a sequel in our concluding remarks and thus reveal the reason for Täckholm's curious findings.

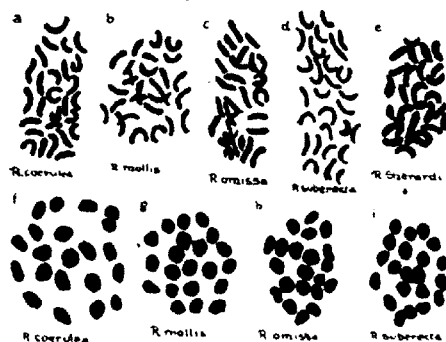
The meiotic phase in these forms is uniform for all, and allowing for its harmony with the same period in *R. Sabini* and *R. coriifolia* one brief description will suffice. It is ushered in by a typical synapsis, opening out

¹ Also known sometimes as the *R. Sherardi* or even the *R. subglobosa* group.

² This paragraph and the next were inserted after we had received a copy of Täckholm's brief paper.

later in the ordinary fashion into an apparently continuous spireme. When this breaks up its units soon reveal themselves as of two distinct types: univalent rods and twisted bivalent loops. This peculiarity was first observed in *Rosa omissa*, and afforded the clue which led to the discovery of the partial reduction in pentaploid and tetraploid alike (Pl. X, Fig. 28 a). At the inception of the spindle the bivalents are sharply marked off from the univalents by their early passage to the equator, and even by separating ere the latter reach it. Here we think it necessary to remark that, in spite of this, equatorial plates with all the chromosomes practically in one plane are much more frequent in tetraploid than in pentaploid roses.

The bivalents diverge to the poles first and form a kind of cap, whilst the others divide and keep together in twos and threes to pass slowly towards them. In the *Villosae* nearly all, and indeed sometimes actually all, attain



TEXT-FIG. 5. Section Villosae. Somatic and heterotype metaphases. $\times 3,000$.

that goal, and are included in the daughter nuclei: occasionally one or two may be left out and yield micronuclei.

The homotype divisions begin in much the same way as the heterotype. Usually, however, only the seven descendants of the bivalents group themselves at the poles, for the others delay their splitting so long that they fail to join them, thereby necessitating the formation of accessory nuclei. If this section manage to keep together, the extra nuclei may have as many chromosomes as, or even more than the normal nuclei formed at the cap, but this occurs only rarely. In general we are inclined to think that nearly always they contain less, and only microspores derived from the cap are capable of growth. The cytoplasm of the multinucleate body (better described as an octad) then segments more or less evenly into eight parts, each containing one or more nuclei.

As demanded by the chromosome counts made during meiosis, every *Villosa* microgene gave a somatic count of twenty-eight (Text-fig. 5. a-e),

and is therefore tetraploid. To all intents and purposes their behaviour agrees with that of the pentaploids and with that of *Rosa Sabini*, although in this case only seven bivalents appear with the fourteen univalents (Text-fig. 5, *f-i*).

V. CONCLUDING REMARKS.

From the foregoing account it appears that in our roses the chromosome number upon which the various complements seem to hinge is seven, and, using this as a base, our local forms arrange themselves as in the appended table:

Diploid.	Tetraploid.	Pentaploid.	Hexaploid.
<i>Rosa arvensis</i> (Systylae) <i>Rosa rugosa</i> (Semiberbaceae)	<i>Rosa pimpinellifolia</i> (two forms, one sterile and the other fertile, as well as <i>R. spinosissima</i>). The whole of the Villosae including <i>Rosa omissa</i> , Déségl. <i>Rosa</i> hybrid <i>pimpinellifolia</i> × (<i>pimpinellifolia</i> × <i>cortifolia</i>).	All the members of the Eucaninae, Afzelianae, Rubiginosae, Tomentosae, and one hybrid, <i>Rosa coriifolia</i> var. <i>Lintoni</i> × <i>Rosa luteoliana</i> .	Hybrid <i>Rosa pimpinellifolia</i> × <i>R. tomentosa</i> var. <i>sylvestris</i> .

As set out in the above table one discrepancy, but that glaring, exists between our results and those of Täckholm; that worker finds *Rosa omissa* to be hexaploid, whereas we determine it to be quite an ordinary tetraploid Villosan. Luckily we are able to explain the disagreement. When satisfying Almqvist's request for British roses we included in the consignment three examples of *Rosa Sabini* (*R. pimpinellifolia* × *R. sylvestris*). All three were returned labelled *Rosa permollis*, Almq. Now, looking up this name in Almqvist's synopsis, we note that it falls under the species type of *Rosa Acharii* and in the section Villosae glauciformes, i. e. with glaucous hairy leaves. Ignoring for a moment that our rose is, without the shadow of a doubt, a *pimpinellifolia* hybrid, this is demonstrably incorrect, although one cannot blame Almqvist for the error. We sent him specimens with the half-open flowers proper to an *inveluta* form, so that its *tomentosa* affinities were masked. Judging from its prickly characters alone he was almost compelled to recognize in it a Villosan. Making the necessary correction for the section, we find that under Almqvist's classification it runs out to *Rosa omissa*, Almq., and thus, as proved from authentic specimens, not of Déséglise.¹

Almqvist's plant is therefore a *pimpinellifolia-tomentosa* hybrid parallel to, if not identical with, our *Rosa Sabini*—a determination explaining the perfect agreement in hexaploidy, and the possession of twenty-eight potential bivalents and fourteen univalent chromosomes, existing between our *R. Sabini* and Täckholm's *R. omissa*.

¹ This paragraph was added after Täckholm's paper came to hand.

Proceeding, let us submit the contents of the table to detailed analysis. The occurrence of diploid, tetraploid, and hexaploid species within the same genus would, at first sight, call for little comment, for intensive work like that of Tahara on *Chrysanthemum* has revealed in that genus comparable numbers based on 9, *Chrysanthemum carinatum* having 9 haploid, *C. leucanthemum* 18, *C. morifolium* 27, *C. Decaisneanum* 36, chromosomes and so on. On the contrary the pentaploid microgenes, comprising fully ninety per cent. of our roses, are quite unexpected, and in view of the very obvious difficulties in the way of their producing a regular haploid number equivalent to half the diploid, their presence and chromosome complement must be regarded as quite anomalous. Nevertheless, a critical study of their microspore development affords some clue to their evolution. However, deviations from the normal do not end with pentaploid roses; when the contents of the column labelled tetraploid are viewed in the light of the above descriptions they present themselves as a very heterogeneous assemblage. There not only do we encounter forms in which the course of pollen formation is quite as even as in *Rosa arvensis* upon which we found our comparisons, but in addition microgenes, appertaining to the Villosae, are included with their cytology in complete harmony with that of the pentaploid roses. For the purposes of this discussion the Villosae may thus be considered with the other subsections of the Caninae, i. e. the Eucaninae, Afzelianae, and the like. Similarly, the hybrid between *pimpinellifolia* and *coriifolia* may be classed with the hexaploid hybrid. Furthermore, although the chromosomes behaving more or less regularly are twenty-eight in the last two plants, and fourteen in the pentaploid species and the Villosae, both groups may be treated as making one whole, since no significant discrepancies exist between the two sets; whatever explains the untypical meiotic phenomena of the one explains it in the other.

We are now in a position to take a comprehensive view of the cytological features emerging from our work. Almost immediately we discover that our local roses conform to two types. Type I, including very few species, pursues a course perfectly normal in all its details. Differing therefore in no wise from other phanerogams at the same stage it requires no further treatment than the above. On the other hand, Type II, comprising the vast majority of our roses, whilst offering differences in detail in its various members, follows the same general but untypical plan throughout. In it we have displayed a heterotype division, equatorial as far as the bulk of its chromosomes is concerned, but reductional with a further fixed proportion, generally fourteen, but sometimes twenty-eight in number.

As is obvious, three recognized hybrids are arranged under Type II, and, as far as behaviour goes, they cannot be severed from their companions. The pairing of their homologous chromosomes in preparation for the heterotype division, and the number doing so successfully, are too suggestive

for that. But in all of their irregular behaviour at this stage—the failure of complete pairing, the lagging of chromosomes both in passing to and from the equatorial plate, the formation of micronuclei, the accentuation of these features in the homotype division, and, finally, the generation of defective pollen—the known rose hybrids differ in no way from what we have pictured for us by Rosenberg as occurring in his *Droserae*, Tischler in his *Bryoniae*, and by ourselves, amongst others, in animal hybrids. We must, therefore, assign the same irregular processes encountered at the same stages in *Rosa* to the same cause, and that cause hybridity. Once having admitted this in the case of the patent rose hybrids, to the same disturbing influence we must look for our explanation of precisely the same unusual occurrences in the other forms; they are assuredly latent hybrids. In this manner, at one stroke, by invoking hybridity we account for the extraordinary phenomenon of pentaploid species, the harmony between their cytology and that of the tetraploid *Villosae*, and lastly, the whole chain of circumstances arising from the peculiarities in their meiosis.

Digby, following Juel, has urged that all of the above-mentioned irregularities, beginning with the untidy spindle figures and culminating with abnormalities of chromatin distribution in the heterotype and the homotype divisions, cannot be regarded as conclusive proofs of hybridity, and instances the classic case of *Hemerocallis fulva* as proving that such behaviour occurs in pure species. To us this seems to savour strongly of begging the question, for not a vestige of proof has ever been adduced to substantiate the claims of *Hemerocallis*, and other plants exhibiting similar divergences from typical microspore formation, to be regarded as 'pure breeds'. On the contrary, if we might be permitted to base our opinions on the definite proofs provided by absolutely the same phenomena detected by Rosenberg in artificial hybrids in *Hieracium* and by ourselves in the patent crosses in *Rosa*, and furthermore by aiding our judgement by evidence derived from other instructive similarities, we feel sure that in the case of the plant treated as the crucial example we have a persistent, if more or less latent, hybrid like our roses.

Comparing the pseudo-reduction in the pentaploid roses and their tetraploid associates, and backing up the comparison with what obtains in *Rosa Sabini*, we are bound to admit that only one satisfactory explanation exists for their common peculiarities. Just as the ten chromosomes derived from *Drosera rotundifolia* find mates in the reduction division of *D. obovata* in ten provided by the *D. longifolia* parent, so one must conclude that the common seven running through the whole *Caninae* supersection has been introduced by some species (quite possibly *Rosa arvensis*) possessing that number as its haploid complement, when it has crossed with a microgene or microgenes with a haploid number of twenty-one in the *Villosae* and twenty-eight in the rest of the *Caninae*. This notion might seem to demand

as a corollary the existence of sexual hexaploid and octoploid roses, if not now, then in some past epoch favouring hybridity.

To postulate sexual forms with such high chromosome numbers would thus seem almost unavoidable were it not that two lines of escape remain open, one in the possible occurrence of repeated back crosses, and the other in mitotic curiosities like the 'mass' homotype (Pl. X, Fig. 40) observed in *Rosa Sabini*.

In this fundamental hybridity itself lies one source of the variability of the Rosae, since in an ordinary F_1 generation of species hybrids quite a wide spread of variation between the conditions of the parents is possible. But in the Rosae the spread in any given form (species?) is far beyond that, and hints at that seen in F_2 generations. We have thus to determine whether any mechanism revealed in the above investigations allows for an approximation to the circumstances of such an F_2 lot.

In all the descriptions we have given we have emphasized the constancy of the appearance of seven pairs of chromosomes in the diakinesis, and of a curious duplication of the subsequent anaphase; the anaphase involving this paired lot occurred long before that in which the remainder took part. So long was the latter delayed that, in many cases, the chromosomes never reached the poles, but either formed micronuclei or were lost in the cytoplasm to degenerate there. On the other hand, the associated seven uniformly massed themselves as a cap at the poles, and accompanied by some of the split univalents formed one of the independent major nuclei visible in the interkinesis. At the homotype division these circumstances are repeated and exaggerated, with the result that when functional pollen is formed, in the great majority of cases, the nuclei of the active grains contain only seven chromosomes, and these seven originating in the seven bivalents of the heterotype division. This was beautifully shown in the large number of counts made during a close study of the fate of these chromosomes in *Rosa coriifolia* (type of Fries).

Since it is merely a matter of chance which member of the original homologous fourteen lies on any given side of the equatorial plate, it appears from this that we have the same mechanism set before us for the segregation of the multitudinous factors of *Rosa* as is used in explaining the segregation of single Mendelizing factors. With seven chromosomes taking part, the number of possible combinations is enormous, so that gametes carrying them must produce in derived zygotes a huge range of genotypes which we see expressed phenotypically in the excessive variation of the roses.

In order that the constancy in the somatic chromosome numbers may be maintained the egg-cell in the Villosae must be endowed with twenty-one chromosomes and in the rest of the Caninae with twenty-eight, exactly as Tackholm has found and as we deduced theoretically. In other words,

when both are functional, the two gametes, the generative nucleus of the pollen grain and egg-cell of the same plant, carry widely different chromosome numbers. Therefore reciprocal crosses between bushes of diverse chromosome complements should result in hybrids with different somatic counts, but when between members of the same group, as in the pentaploid section, they should agree and both be pentaploid. In the only bush of the latter type examined the somatic number was thirty-five as demanded by theory. Moreover, as the bush grew adjacent to and resembled *Rosa coriifolia* var. *Lintoni*, it was a fair inference that that microgene had been the seed parent. In addition, since the other roses growing near by were all *Rosa lutetiana*, and in certain of its characters and ontogeny that form was approximated, *Rosa lutetiana* in all probability had acted as pollen parent. Now the egg-cell of *Rosa coriifolia* var. *Lintoni* would contain a nucleus with twenty-eight chromosomes, and the generative nucleus of *lutetiana* pollen would probably possess seven, so that if these numbers have any value in determining the affinities of the hybrid it should resemble *Lintoni* more closely. Such was indeed the case, the *lutetiana* influence appearing mainly in the styles, stigmas, sepals, and young shoots, and less markedly in the prickles and glandular vestiture.

Similarly, in the case of the *Rosa Sabini*, if *Rosa sylvestris* were the seed parent, then the hybrid should possess $14 + 28 (= 42)$ chromosomes; if, on the other hand, it were the pollen parent, the hybrid number ought to be $14 + 7 = 21$. Its somatic number was 42, whence we deduce that almost certainly the first conjecture was true, as was confirmed, firstly, by the fact that the bush in question grows in hedges amongst *Rosa sylvestris* and far from the nearest *Rosa pimpinellifolia*, and, secondly, it resembles *Rosa sylvestris* very much more closely than it does *R. pimpinellifolia*.

The case of the *Rosa* hybrid *pimpinellifolia* \times (*pimpinellifolia* \times *coriifolia*) is much more difficult to explain. If *Rosa pimpinellifolia* were the seed parent, as seems likely from the fact that the hybrid grows in a *pimpinellifolia* colony on the Northumberland sand dunes, and fully 100 yards from the nearest *coriifolia*, the chromosome number should be $14 + 7 = 21$; if the reverse held true, it ought to be $28 + 14 = 42$. Neither figure is correct, the somatic count being 28.

Now an egg-cell of *R. pimpinellifolia* with fourteen chromosomes must form the starting-point, if we judge from the habitat of the plant. Suppose this to be fertilized by *Rosa coriifolia* pollen with a nucleus endowed with seven chromosomes. The hybrid would thus display $14 + 7 = 21$. In its megaspore formation, should events follow the usual course of *Rosa* hybrids, seven chromosomes would be shed, leaving an egg-cell with fourteen. As it is a matter of chance how the chromosomes are arranged in the partial reduction division, this fourteen would originate partly with *coriifolia* and partly with *pimpinellifolia*. Now for the pollination of this

rose to take place, situated as it would be amidst an abundance of *R. pimpinellifolia*, other than by the agency of that rose appears a very remote contingency. Thus an egg-cell nucleus with fourteen chromosomes would fuse with that of a pollen grain with fourteen, giving us a zygote with $14+14 (= 28)$ such as we have.

In this manner, our hybrid reveals itself as a back cross between *R. pimpinellifolia* and a hybrid *pimpinellifolia* \times *coriifolia*. Strangely enough it betrays its hybrid nature in the same characters as the *coriifolia-lutetiana* cross, i. e. in styles, stigmas, sepals, and prickles.

From this we gather that the chances are great that reciprocal rose crosses should differ as stated above, and that they should be preponderatingly matroclinous.

As we have demonstrated experimentally (Harrison, 1920), certain of the Villosae, Afzelianae, Rubiginosae, and Agrestes set seed by some process of apomixis. Moreover, we have likewise proved this to be of the facultative order, since seedlings showing hybrid characters have originated from flowers, pollinated with foreign pollen, growing on the same bush as those castrated, and producing seeds apomictically. Despite this, in the sections just named, our experiments lead us to the opinion that in their case apomixis is the rule and sexual reproduction the exception. On the contrary, with the *lutetiana* and *dumetorum* allies, as well as with certain Villosae, our work indicates with some certainty that, whilst they are too facultatively apomictical, they favour pollination—a view not incompatible with occasional apomixis, as the work of Pace has proved. However, in any case, the difference is merely one of degree, and the situation is best summed up by stating that except for *Rosa arvensis*, *R. rugosa*, and *R. pimpinellifolia* all the roses brought under examination exhibit apomixis.

This must not be assumed to be simple parthenogenesis, because, as we have explained, the egg-cell in the Villosae has only twenty-one chromosomes and in the rest of the super-section Caninae twenty-eight. Should these cells develop without fertilization the somatic numbers in the embryos thus generated must necessarily be twenty-one and twenty-eight respectively, which is emphatically not the case. Judging from the uniform chromosome number of twenty-eight in the Villosae and of thirty-five in the Afzelianae, &c., we must conclude that, whatever cell produces the new organism, it must be somatic in origin and therefore possess the chromosome number proper to such cells.

Now let us assemble our facts. We have decided that, whilst the sexual roses are genetically pure, the non-sexual (or occasionally sexual) microgenes are hybrid in nature. Apparently, therefore, some intimate connexion exists between hybridity and apomixis. Many authors following Rosenberg and Strasburger have seen in the mere size of the chromosome numbers in plants like the Alchemillae and Antennariae a cause of apogamy,

and others, represented by Gates, by a refined extension of the crude notion put forward by previous writers have been inclined to see in tetraploidy and octoploidy the inciting cause. With neither of these do we agree. We see in our particular form of vegetative reproduction, and in the true apogamy of other species, a further manifestation of the powers of heterosis stimulating, in the one case cells from the soma, and in the other the germ-cells, to an enormously exaggerated development ending in apomictical reproduction. High chromosome numbers are in this view merely the attendant circumstances of the hybridity and the stimulus induced by it.

It needs only a glance to perceive the close analogy between the cytology of roses and that of other forms betraying apogamy or some kindred form of reproduction. Our roses behave exactly like Rosenberg's *Hieracia*, Holmgren's *Eupatoria*, and Ernst's *Chara crinita*. In all of these cases, as in others, the apogamy is assigned to hybridity, and we feel sure that these authors are justified in the position assumed. To our minds apogamy and the enormous variability in *Rosa* and other critical genera like *Hieracium*, *Taraxacum*, *Antennaria*, and *Callitriche* alike take their origin from one and the same cause, hybridity—not set in motion now, but in the far distant past.

Whatever value we assign to the various rose forms, and this, in spite of their hybrid nature, cannot be assessed at less than the microgene or Jordanian species, their development became possible through crossing. Moreover, despite the vast range of variation, the centre round which any given microgene oscillates remains fixed; no one, for instance, could ever mistake a member of the *Rosa lutetiana* fraternity for a *R. glauca* form and so on. Undoubtedly, therefore, hybridity has given rise to these recognizable, if variable, units just as to the more stable *Erigeron annuus*, so that hybridity must be admitted as one of the prime factors in the evolution of species, if not the sole one as Lotsy would fain persuade us.

Further, if, for example, we can imagine the egg-cell of *Rosa Borreri* to be fertilized by a pollen grain built up from the seven 'cap' chromosomes of *R. omissa*, or any other similar cross pollination, it appears far from impossible that some form of microgene building is still proceeding. If apomictical, as seems likely when one remembers the Lord Penzance hybrid Rubiginosa, the new form would be reasonably permanent from the very first. We feel certain that the forms arranged under *Rosa subcanina*, *R. subcollina*, and other groups are comparatively recent developments rendered possible by the northern forms belonging to the Afzelianae clashing with the more southern Eucaninae, possibly at the onset of the Glacial Period, whilst others, like *R. foetida* and *R. andagavensis* allies, may even yet be coming into being.

Lastly, we propose to consider the taxonomic value of our observations.

Since our conceptions of the status of the British roses, derived from the work outlined above and otherwise, differ profoundly from those held up to the present by most, but certainly not all, rose specialists, the ground is not prepared for any definite utilization of the observed chromosome numbers. Notwithstanding this one cannot help being struck by the fact that all of the supersection Caninae, minus the Villosae, are pentaploid forms, which confirms an alliance between the more widely separated *R. tomentosa* and *R. foetida* groups on the one hand, and the Eucaninae on the other, suggested by many similarities more apparent on the growing bushes. In much the same manner the agreement in tetraploidy between the obviously related *R. mollis*, *R. omisssa*, and *R. Sherardi* forms serves to strengthen the case for our previous removal of the whole of these groups to the Villosae. Hence we see that the old classification of *R. omisssa* and *R. Sherardi* as *tomentosa* segregates, upon no more secure basis than the non-persistence of their sepals, fails both when judged from a cytological standpoint and from their undoubted approximation in general characteristics when one examines them otherwise than as dried fragments.

SUMMARY.

1. The fundamental chromosome number in *Rosa* is seven.
2. Among the roses examined were diploid, tetraploid, pentaploid, and hexaploid forms.
3. No diploid form was found to be abnormal during meiosis; nor was any member of the *pimpinellifolia* section among the tetraploid.
4. The remainder of the tetraploids, the whole of the pentaploids and hexaploids, showed a partial reduction involving fourteen or twenty-eight chromosomes.
5. In these partially reduced forms the heterotype anaphase occurred in two steps, one involving the chromosomes to which we have just referred, and the second, and later, taking place when the univalents split.
6. In many cases these split univalents failed to reach the poles and formed supernumerary micronuclei in the interkinesis between the heterotype and homotype divisions.
7. The features mentioned in 5 and 6 are exaggerated in the homotype division, so that, for the most part, the major nuclei in the tetrad are endowed with seven chromosomes in the pentaploids and abnormal tetraploids.
8. When the remainder of the chromosomes keep in two groups, eight major nuclei may arise, thus yielding an octad rather than a tetrad.
9. Multinucleate pollen grains are quite common in the anomalous forms of the genus *Rosa*, but when these are derived chiefly from micronuclei the pollen grains collapse.

10. Known hybrids showed the same type of behaviour as outlined in paragraphs 4-9.

* 11. Arguing from points 4-10 we have decided that every rose studied showing partial reduction was really of hybrid origin.

12. All the abnormal roses are facultatively apomictical; this we assign to the stimulus of heterozygosis.

13. Hybridity, in this view, is likewise responsible for the wide range of variation in the genus *Rosa*.

14. We have shown that hybridity is one of the sources of microgene building in the genus *Rosa*.

15. This process of microgene building is probably still proceeding.

16. Since the functional pollen grains in the supersection *Caninae* contain seven chromosomes and the egg-cell twenty-one in the *Villosae*, and twenty-eight in the rest of the *Caninae*, it is clear that reciprocal hybrids between the two named will have different chromosome numbers.

17. Crosses within the limits of the pentaploid and abnormal tetraploid forms will have the same number of chromosomes as their parents, but, as in the crosses referred to in 16, will be strongly matroclinous.

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Rose Forms as determined by their Cytological Behaviour. 187

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EXPLANATION OF PLATES IX AND X.

Illustrating Miss Blackburn and Dr. Harrison's paper on the Status of the British Rose Forms.

PLATE IX.

Illustrating Meiosis in the Pollen Development and the Somatic Mitoses in the Normal Types of *Rosa*.

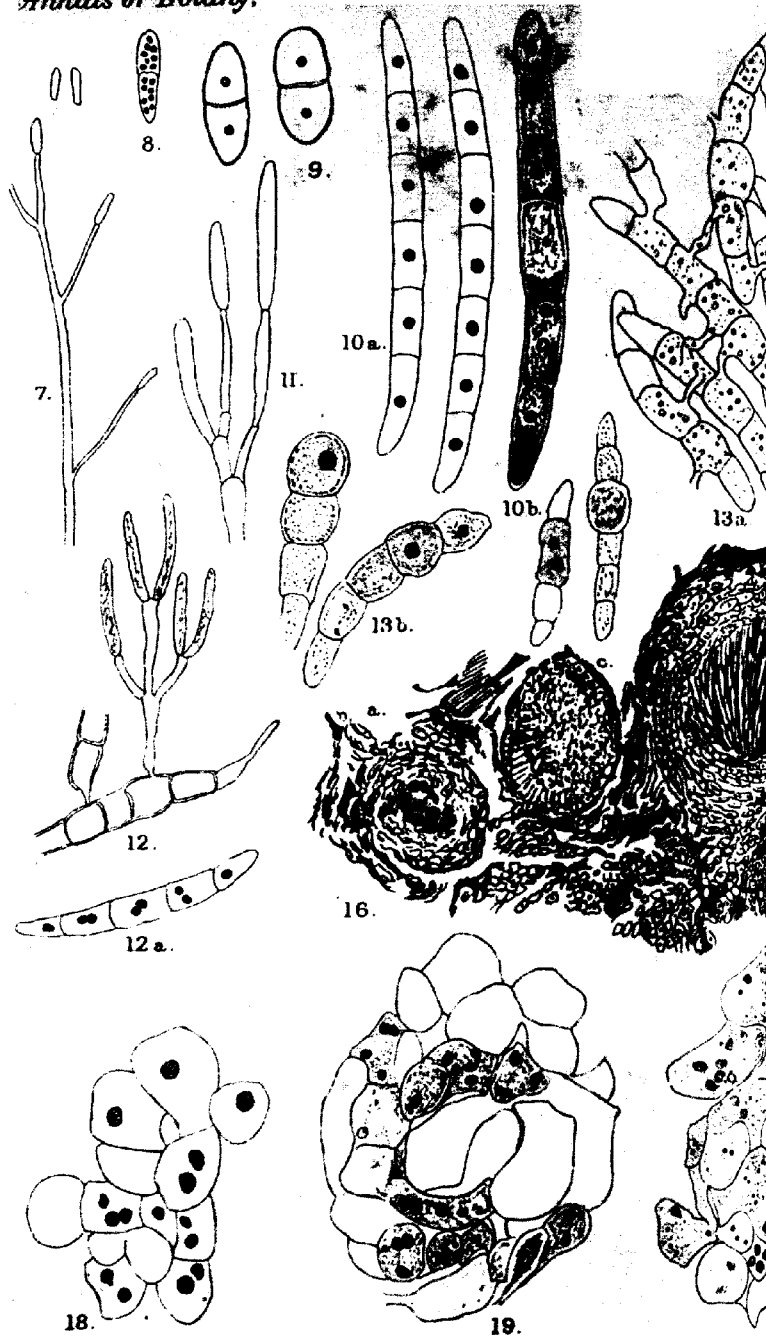
Figs. 1-3, 7, 10-15, and 18-22 from *Rosa arvensis*.
Figs. 4-6, 8-9, 16, and 22-25 from *Rosa pimpinellifolia*, and Fig. 17 from *Rosa rugosa*.
All the figures were drawn with the camera lucida. Mag. 4,000.

- Fig. 1. Pollen mother-cell previous to synapsis.
Fig. 2. Synapsis.
Fig. 3. Evolution of spireme from the synaptic knot.
Fig. 4. Spireme completely expanded.
Fig. 5. Bivalent units of spireme showing longitudinal split.
Fig. 6-9. Stages in development of the bivalents.
Fig. 10. Diakinesis.
Fig. 11. Multipolar spindle stage.
Fig. 12. Metaphase of the heterotype division.
Fig. 13. Equatorial plate of *R. arvensis* showing seven chromosomes.
Fig. 14. Telophase.
Fig. 15. Interkinesis after heterotype division.
Fig. 16. Metaphase of the homotype division.
Fig. 17. Anaphase from *R. rugosa*.
Fig. 18. Telophase of the homotype division.
Fig. 19. Completed tetrad.
Fig. 20. The four spores of the tetrad separated.
Fig. 21. Late somatic prophase.
Fig. 22. Somatic equatorial plate showing 14 chromosomes.
Fig. 23. The same in *R. pimpinellifolia* showing 28 chromosomes.
Fig. 24. Somatic metaphase.
Fig. 25. Late somatic anaphase.

PLATE X.

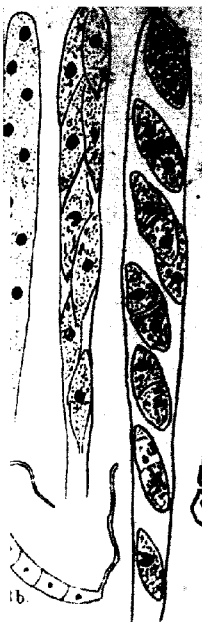
- Fig. 26. Synapsis in *R. Sabini*.
Fig. 27. Spireme in *R. Sabini*.
Fig. 28. Differentiation of bivalent loops and univalent rods in *R. pratinensis*.

- Fig. 28 a. Slightly later stage from *R. omisssa*.
 Fig. 29. Diakinesis in *R. Sabini*, showing some bivalents.
 Fig. 30. Bivalents on the spindle univalents still scattered from *R. coriifolia*.
 Fig. 31. Complete metaphase in *R. rubiginosa*.
 Fig. 32. Tripolar spindle in *R. rubiginosa*.
 Fig. 33. Telophase of bivalents, anaphase of univalents, in *R. Sabini*.
 Fig. 34. The same end on, showing the lateral position of the spindle in the mother-cell.
 Fig. 35. Slightly later stage in (*R. pimpinellifolia* \times *coriifolia*) \times *pimpinellifolia*.
 Fig. 36. The same in *R. coriifolia*.
 Fig. 37. Interkinesis in *R. Sabini*.
 Fig. 38. Metaphase of the homotype division in *R. Sabini*.
 Fig. 39. Anaphase of homotype in *R. coriifolia*.
 Fig. 40. Curious double spindle from *R. Sabini*.
 Fig. 41. Interkinesis after the homotype showing numerous nuclei in *R. coriifolia*.
 Fig. 42. Octad from *R. rubiginosa*.
 Fig. 43. Young pollen grain from *R. sylvestris* showing three nuclei.
 Fig. 44. Defective pollen grains with normal from *R. flexibilis*. \times 250.
 Fig. 45. Somatic anaphase from *R. sylvestris* to show absolute regularity.

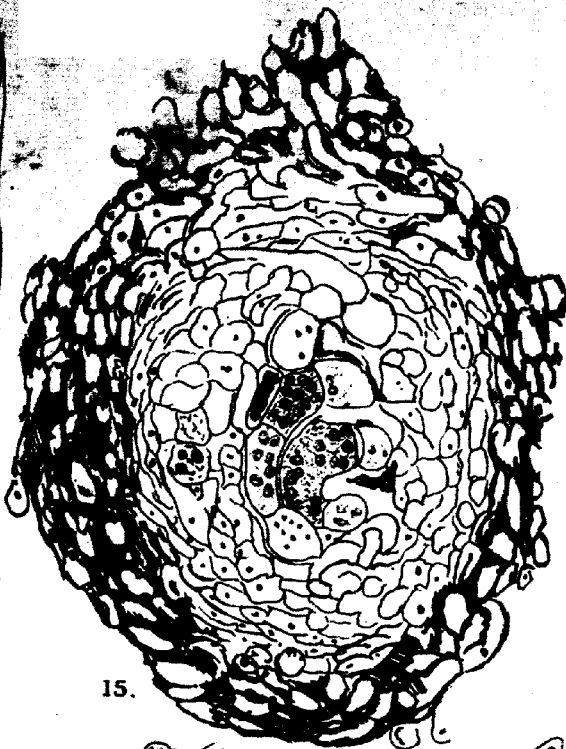


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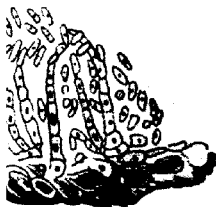
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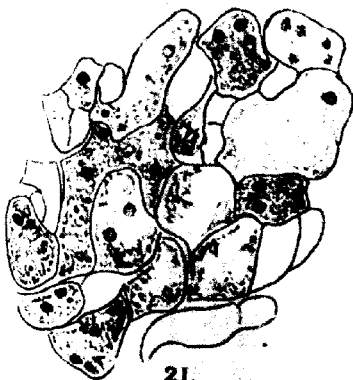
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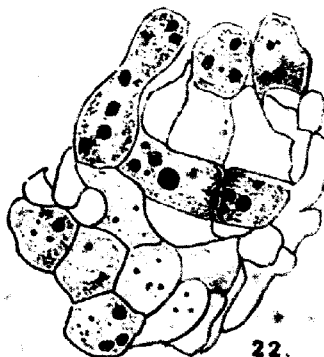
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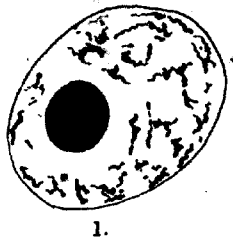
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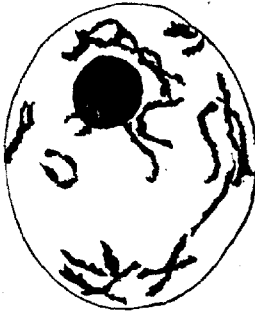
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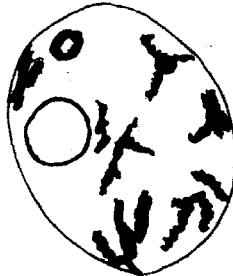
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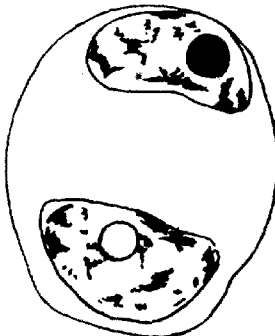
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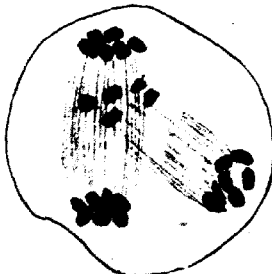
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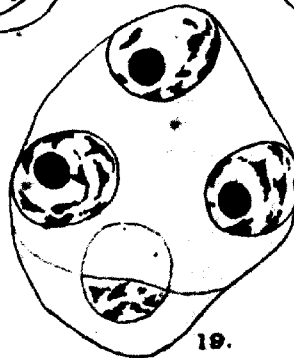
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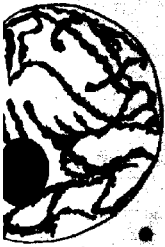
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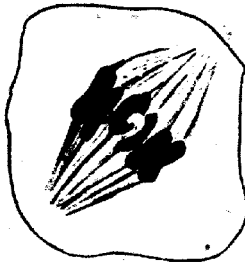
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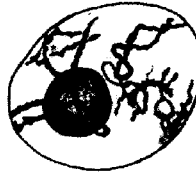
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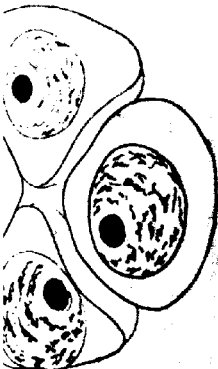
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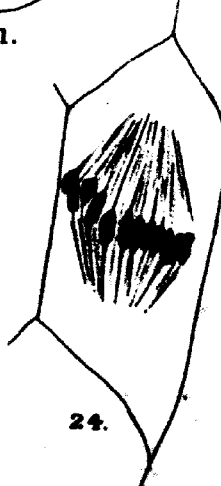
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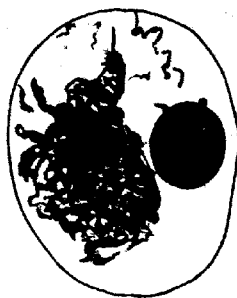


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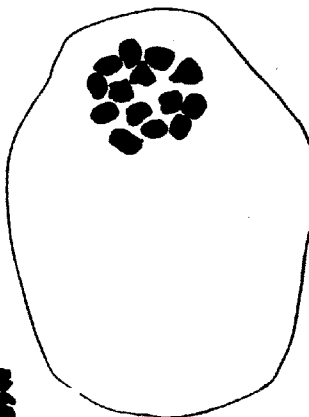
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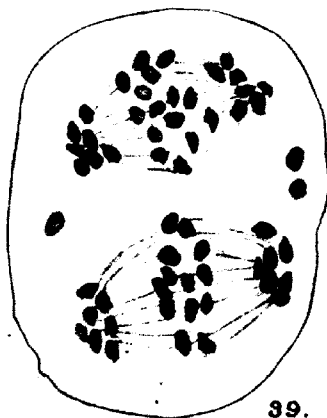
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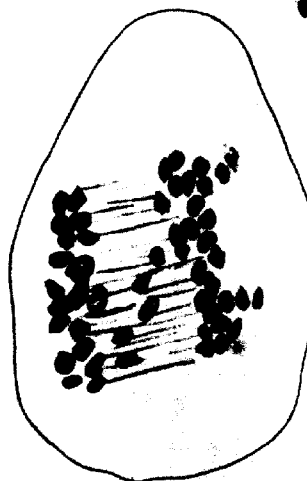
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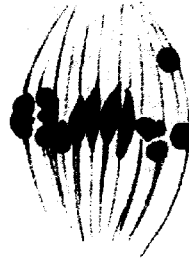
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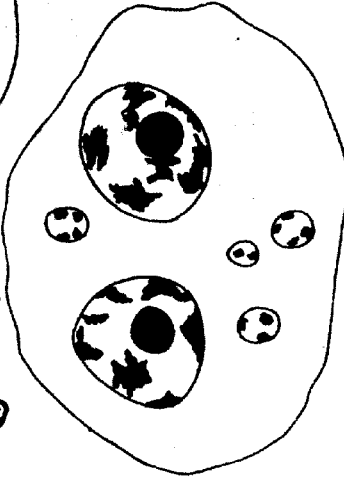
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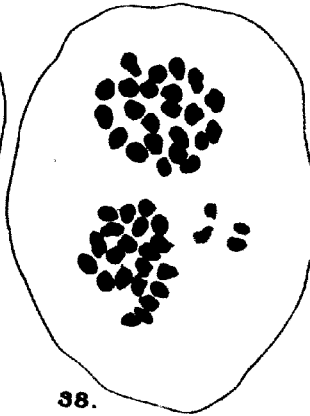
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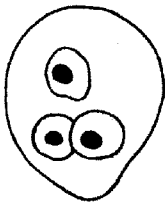
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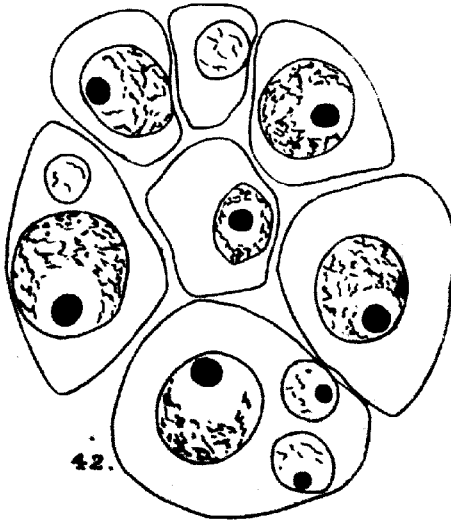
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42.

Relation of Potassium to Growth in Plants.

BY

T. O. SMITH

AND

O. BUTLER.

IN the present study we propose to determine, first, the effect of absence of potassium on the growth of plants, and, second, to what extent recovery is possible when potassium is supplied after more or less prolonged growth in its absence.

The effect of absence of potassium on growth has been studied by several authors, but little attention has been given to the question of recovery from potassium starvation. Nobbe, Schroeder, and Erdmann¹ have studied the effect of potassium on the growth of Japanese buckwheat and summer rye. In the experiment with buckwheat the authors studied not only the effect of absence of potassium on growth, but also the effect produced by an addition of the element on the extent and rate of recovery from starvation of plants already much enfeebled. In the experiment with summer rye only the effect of absence of potassium on growth was studied.

Lüpke² studied the effect of potassium on the growth of *Phaseolus multiflorus* and *P. vulgaris*, and incidentally the effect of delayed additions of the element on recovery.

Wilfarth³ studied the effect of absence of potassium on the growth of barley, oats, lupins, and peas, and, in collaboration with Wimmer⁴—considerable attention being at the same time devoted to the symptoms of potassium starvation—studied potato, tobacco, buckwheat, mustard, chicory, oats, and sugar beets. The experiments in all cases showed that potassium was

¹ Nobbe, F., Schroeder, J., and Erdmann, R.: Ueber die organische Leitung des Kaliums in der Pflanze. Landw. Versuchs-Stat., xiii, 321-99, 401-23, 1871.

² Lüpke, R.: Ueber die Bedeutung des Kaliums in der Pflanze. Landw. Jahr., xvii, 867-913, 1888.

³ Wilfarth, H.: Vegetationsversuche über den Kalibedarf einiger Pflanzen. Arbeiten der deutschen Landwirtschaftsgesell., xxxiv, 101 pp., 1898.

⁴ Wilfarth, H., and Wimmer, G.: Die Wirkung des Kaliums auf das Pflanzenleben. Ibid., lviii, 106 pp., 1901.

essential to growth, and that the symptoms of potassium starvation, allowing for individual idiosyncrasies, were the same in all the plants.

The results which we have in turn obtained with wheat, corn, and buckwheat will confirm the findings of previous workers, and especially emphasize the importance of potassium being present at an early stage in the growth of the plant.

I. THE EFFECT OF ABSENCE OF POTASSIUM ON GROWTH.

In our study on the effect of absence of potassium on growth, two experiments were carried out with wheat and corn, respectively, and one with Japanese buckwheat.

Experiment 1. In this experiment Blue stem Wheat was grown twenty-one days in water culture. The plants were obtained from seed previously carefully selected for size and weighing not less than 20 mg. nor more than 35 mg. An analysis of several samples of this selected seed, containing from 800 to 1,400 seeds per sample, showed that the mean potassium content of a single seed was 0.11 mg. The seeds were germinated on paraffined cork sheeting 2 mm. thick, checkered with holes, which was floated on distilled water in a moist chamber also paraffin coated. The seeds were placed upon the cork with basal end protruding over the holes and the embryo facing upwards, so that upon germination the normal direction of growth was immediately assumed. This precaution made easier the transfer of the seedlings to the culture solutions, since the transfer was made when the plants were very young and it was essential that all the roots developed be immersed. Three days after the seeds were placed in the germinator they had grown sufficiently to permit transference to the culture solutions. The culture solutions were contained in glass jars 10 cm. in diameter and 30 cm. high, containing approximately 1,600 c.c. The jars were said by the manufacturer to have been made from sand, soda-ash, and lime. Samples taken for analysis showed that the glass had a mean potassium content of 0.66 per cent. The jars were covered with paraffined linen securely tied over the top as support for the plants. Six holes were punched in the linen, five equally spaced for the plants and the sixth excentrically for a tube 12 mm. in diameter and 25 cm. long, through which the water lost was added as required. In the preparation of the nutritive solutions only chemically pure salts were used, and they were all carefully examined spectroscopically for potassium, no more than a trace of this element being allowed. Care was also taken in the preparation of the distilled water to keep it free from potassium.

In deciding on the type of nutritive solution to use we had first of all to select one that not only grew vigorous and healthy plants, but one that could also be modified so as to exclude potassium without any serious

change occurring in concentration or elemental composition. We therefore chose for our working basis a full nutritive solution that has been much and very successfully used at Rothamstead.¹ The composition of this solution is given in Table I so that the reader may properly appraise the changes that we found it necessary to introduce. The type of full nutrient (solution A) that we have used was prepared as shown in Table II. The difference between solution A and the Rothamstead solution lies in the elemental composition, not the concentration. Solution A contains more calcium and nitrogen, but less sodium and chlorine. The increase in the amount of calcium used causes the calcium-magnesium ratio to change from 2.3 : 1 to 3.8 : 1, but the calcium-magnesium ratio can, as we shall see, be changed even to a greater extent without injury resulting; and the reduction in sodium and chlorine cannot be considered a matter of great importance since plants may be successfully grown over considerable periods of time in their absence.

The nutritive solution less potassium was prepared by substituting calcium nitrate for potassium nitrate on the basis of the nitrogen content of the two salts and adding calcium sulphate in the amount required to bring up the concentration to 3.04 parts per litre. The method of preparation and the elemental composition of this solution (solution B) are given in Table III. It will be noticed that the nutritive solution less potassium has the same concentration as solution A, and, with the exception of the potassium, differs in elemental composition from it only in greater richness of calcium and sulphur. The calcium-magnesium ratio is, therefore, also changed, and instead of being 3.8 : 1 as in solution A, has become 10.9 : 1. Owing to the importance that has been attached to the calcium-magnesium ratio, we did not feel justified in carrying the experiment through without at the same time verifying the fact that the effect on growth of solution B could not be attributed to the change in ratio introduced. We therefore prepared a full nutritive solution (solution C) containing a calcium-magnesium ratio of 10.9 : 1. The method of preparation and elemental composition of this solution are given in Table IV. It will be immediately noticed that solution C has a concentration of 1.18 parts per litre greater than either solutions A or B, and that the sulphur content is very nearly twice that of solution B. Solution C, however, possesses the same elemental composition as solution A, with the exception of the elements calcium and sulphur, which are present in larger amounts, and, unless the increase in sulphur or the change in the calcium-magnesium ratio has a disturbing influence, should produce plants in all points similar.

The experiment was begun on August 18, and on September 18 the plants were removed and the roots and tops separated, the plants from each

¹ Hall, A. D., Brechley, W. E., and Underwood, L. M.: *The Soil Solution and the Mineral Constituents of the Soil*. Phil. Trans. Royal Soc., Ser. B, civ, 1914.

TABLE I. Salts used in the preparation of and elemental composition of the Rothamstead solution.

Salts used.	Concentration per litre. Grm.	Elemental composition.										
		K	Mg	Ca	Na	N	S	P	Fe	Cl	O	H
KNO_3	1.00	0.3867	0.1386	0.4747	0.0286
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.50	...	0.0493	0.0651	0.3570	0.0074
CaH_2PO_4	0.50	0.1437	0.1967	0.1139	...	0.3033	0.2350	...
NaCl	0.50	0.2788	0.0018
$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$	0.50	0.1164	0.0931	...	0.0083	0.0157	0.0143	0.0018
$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	0.04	0.0083	0.0157	1.3597	0.0396
Total	3.04	0.5304	0.0493	0.1164	0.1967	0.1386	0.1582	0.1139	0.0083	0.3190

TABLE II. Salts used in the preparation of and elemental composition of nutritive solution A.

Salts used.	Concentration per litre. Grm.	Elemental composition.										
		K	Mg	Ca	Na	N	S	P	Fe	Cl	O	H
KNO_3	1.3716	0.5304	0.1901	0.6311	0.0286
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.5000	...	0.0493	0.0651	0.1139	0.3570	0.0111
$\text{CaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$	0.4031	0.0736	0.1003
NaCl	0.1653	0.1164	0.0650	0.2788	0.0117
$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$	0.5000	0.0931	...	0.0083	0.0157	0.0143	0.0018
$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	0.0400	0.0083	0.0157	1.3597	0.0396
Total	3.0100	0.5304	0.0493	0.1190	0.0650	0.1901	0.1582	0.1139	0.0083	0.1160

TABLE III. Salts used in the preparation of and elemental composition of nutritive solution B.

Salts used.	Concentration per litre.	Elemental composition.									
		Mg	Ca	Na	N	S	P	Fe	Cl	O	H
$\text{Ca}(\text{NO}_3)_2$	1.1131	...	0.2719	...	0.1901	0.6311	...
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.5000	0.0493	0.0651	0.3570	0.0186
$\text{CaH}_2(\text{PO}_4)_2 \cdot \text{H}_2\text{O}$	0.4631	...	0.0736	0.1139	0.2645	0.0111
NaCl	0.1653	0.0650	0.1003
$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$	0.5000	...	0.1164	0.0931	0.2788	0.0117
$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	0.0400	0.0083	0.0157	0.0142	0.0018
CaSO_4	0.1585	...	0.0761	0.0609	0.1215	...
Total ...	3.0400	0.0493	0.5380	0.0650	0.1901	0.2191	0.1139	0.0083	0.1160	1.6871	0.0532

TABLE IV. Salts used in the preparation of and elemental composition of nutritive solution C.

Salts used.	Concentration per litre.	Elemental composition.									
		K	Mg	Ca	Na	N	S	P	Fe	Cl	O
KNO_3	1.3716	0.5304	0.1901	0.6511
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.5000	...	0.0493	0.0651	0.3570
$\text{CaH}_2(\text{PO}_4)_2 \cdot \text{H}_2\text{O}$	0.4631	0.0736	0.1139	0.2645
NaCl	0.1653	0.0650	0.1003	...
$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$	0.5000	0.1164	0.0931	0.2788
$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	0.0400	0.0083	0.0157	0.0018
CaSO_4	1.1819	0.3480	0.2784	0.5555
Total ...	4.2219	0.5304	0.0493	0.5380	0.0650	0.1901	0.4366	0.1139	0.0083	0.1160	2.1211

jar being considered a unit. Thirty-five plants were grown in each of solutions A and B, while twenty plants only were grown in solution C. The jars containing the solutions were alternately arranged so as to compensate for all differences in growth that might result from irregularities in the environment. Wet sawdust was packed around the jars and brought nearly to a level with the tops in order to prevent undue variations of temperature in the solutions and keep the roots in quasi-darkness.

Ten days after the seedlings were placed in the solutions the effect of absence of potassium on growth was already well marked. The leaves of the plants in solution B were drying up at the tips, and small crinkled areas were distributed irregularly on the older leaves; while the plants in solutions A and C were growing nicely and showed no pathognomonic symptoms whatsoever. After sixteen days the plants in the full nutritive solutions began stooling, while the plants growing in the absence of potassium had ceased developing. There was no evidence of stooling and all the leaves were drying up from the tips, the older showing in addition promiscuous dead and dying areas in other portions of the blade. There was no apparent effect upon the chlorophyll due to the absence of potassium, the death of the tissues not being accompanied by any progressive change of colour.

After twenty-one days the plants in the full nutritive solutions appeared to be alike in all particulars; they were healthy and growing vigorously. The plants possessed on the average three stems and a well-developed glistening, white, fibrillose root system. The plants growing in solution B had, on the other hand, evidently ceased all development; the leaves were all withering from the tips, the older leaves showing, as on the sixteenth day, dead and dying areas scatteringly distributed over the blade, but the plants had become increasingly feeble. The root system was poorly developed and the side roots, while numerous, were short and stubby and dull white in colour. At the close of the experiment, therefore, the plants in solutions A and C show no discernible differences, but the effect of the absence of potassium on curtailment of growth was extremely striking. A study of the data given in Table V shows that solution C gave substantially the same growth as solution A. Hence an addition of calcium greatly in excess of that present in solution A does not introduce a disturbing factor, and we may confidently assume that the symptoms exhibited by the plants grown in the absence of potassium were due to the absence of potassium and not to disturbances introduced by the change in the calcium-magnesium ratio or as a result of the increased quantity of calcium and sulphur offered the plants. The mean dry weight of a plant grown in solution A was 0.1631 grm., in solution C 0.1819 grm., a difference of 10.9 per cent., and this difference is still shown when we consider the growth of tops and roots separately, but the relative development of the roots, on the

other hand, is slightly better in the plants grown in solution A. When we study the effect of absence of potassium on growth it makes very little difference, therefore, whether we compare the growth of the plants in solution B with the growth of those in solution A or solution C, though, since solutions B and C are more nearly alike in composition—except for the essential difference, absence of potassium—than solutions A and B, it is presumptive that the growth obtained in solutions B and C more accurately measures the effect of absence of potassium. Plants

TABLE V. *Green and dry weights of Blue stem Wheat after 21 days' growth in nutritive solutions A, B, and C.*

Nutritive solution used.	No. of plants.	Total weight of plants.		Weight of tops.	Weight of roots.	Ratio of tops to roots.
		Green.	Dry.			
		Gm.	Gm.	Gm.	Gm.	Gm.
Nutritive solution A	5	5.8102	0.6256	0.4704	0.1552	3.03
	5	6.9992	0.7099	0.5295	0.1804	2.93
	5	10.1212	0.8659	0.6159	0.2500	2.46
	5	7.9997	0.8016	0.5839	0.2177	2.68
	5	10.9833	1.0473	0.7718	0.2755	2.80
	5	9.3301	0.9029	0.6668	0.2361	2.82
	5	7.3533	0.7558	0.5523	0.2035	2.71
Mean	1	1.6713	0.1631	0.1197	0.0434	2.77
Nutritive solution C	5	9.6943	0.9671	0.7096	0.2581	2.75
	5	4.5958	0.4923	0.3635	0.1288	2.82
	5	7.9318	0.9172	0.6984	0.2188	3.19
	5	11.8819	1.2601	0.9534	0.3067	3.11
Mean	1	1.7052	0.1819	0.1362	0.0456	2.97
Nutritive solution B	5	1.0468	0.1522	0.1264	0.0258	4.89
	5	0.9567	0.1622	0.1347	0.0275	4.89
	5	0.8126	0.1413	0.1128	0.0285	3.96
	5	0.8394	0.1445	0.1197	0.0248	4.83
	5	0.8022	0.1449	0.1214	0.0235	5.16
	5	0.9127	0.1472	0.1194	0.0278	4.29
	5	1.4115	0.2169	0.1671	0.0498	3.34
Mean	1	0.1938	0.0317	0.0258	0.0059	4.48

growing in the absence of potassium, even over the short period of time of twenty-one days, produce between 5.1 and 5.7 times less total dry matter than plants growing in a full nutritive solution; in the case of the tops the loss lies between 4.6 and 5.2, and in the case of the roots between 7.3 and 7.7. Absence of potassium curtails more markedly the growth of roots than the growth of tops. In the full nutritive solutions the ratio of top growth to root growth lies between 2.77:1 and 2.97:1, while in the case of the plants growing in the absence of potassium it is 4.48:1.

If we pass now to a consideration of the relative absorption of and distribution of potassium in the plants we will obtain the results shown in Table VI. The data show that each plant growing in solution B absorbed 0.9 mg. potassium from the solution, a negligible amount. The plants may be considered as having grown in the absence of potassium. The potassium absorbed by the plants growing in solutions A and C is practically identical, the increased concentration of calcium and sulphur in the latter having had no disturbing effect. It will be noticed that in all the plants, irrespective of the solution in which grown, the relative distribution of potassium in tops and roots is the same and is entirely independent of the amount absorbed. On the other hand, the total amount of potassium absorbed per gramme of dry matter formed is dependent on the amount available: the potassium requirement for maintenance and the potassium requirement for growth are not the same.

TABLE VI. *Distribution of and amount of potassium in Blue stem Wheat plants grown 21 days in nutritive solutions A, B, C.*

Nutritive solution used.	No. of plants.	Amount of potassium in			Potassium utilized per gramme dry matter formed.		Ratio of potassium content of tops to roots.		Total potassium absorbed from the nutritive solution by one plant.	
		Tops.	Roots.	Entire plants.	Tops.	Roots.				
		Mg.	Mg.	Mg.	Mg.	Mg.			Mg.	Mg.
Nutritive solution A	35	311.20	104.00	415.20	74.26	68.49	1.08	71.73	11.76	
Nutritive solution C	20	80.10	56.50	136.60	66.09	61.92	1.06	65.05	11.72	
Nutritive solution B	35	5.80	1.20	7.00	6.43	5.78	1.11	6.31	0.09	

Experiment 2. In Experiment 1, it will be remembered, we found it necessary, in preparing the nutritive solution less potassium (solution B), to increase the calcium-magnesium ratio from 4:1:1 to 10:9:1. This was accomplished by simply adding calcium sulphate, and while no disturbing effect was produced, as we have seen, we nevertheless thought it desirable to determine whether one could equally satisfactorily prepare a less potassium solution of the same concentration and same calcium-magnesium ratio as solution A by simply reducing the amount of calcium sulphate used and increasing the amount of magnesium sulphate without introducing any disturbing effect. With this purpose in view we set up Experiment 2, using solution A and two new solutions modified in the manner indicated: a full nutritive solution (solution D) and a less potassium solution (solution E). Thirty plants of Blue stem Wheat were grown in each of the nutritive solutions A, D, and E. Five plants were grown in a jar and the seedlings were placed in the solutions when three days old.

TABLE VII. Salts used in the preparation of and elemental composition of nutritive solution D.

Salts used.	Concentration per litre.	Elemental composition.										
		K	Mg	Ca	Na	N	S	P	Fe	Cl	O	H
	Gram.	Gram.	Gram.	Gram.	Gram.	Gram.	Gram.	Gram.	Gram.	Gram.	Gram.	Gram.
KNO ₃	1.3716	0.5304	0.1901	0.6511
MgSO ₄ · 7H ₂ O	0.5000	0.0493	0.0651	0.3579	0.0386*	...
CaH ₂ (PO ₃) ₂ · H ₂ O	0.4631	0.0736	0.1139	0.3579	0.0111	...
NaCl	0.1053	0.0650	0.1003
CaSO ₄ · ½H ₂ O	0.5000	...	0.1164	0.0931	0.3788	0.0117	...
CaSO ₄ · H ₂ O	0.5000	0.0083	0.0157	0.0143	0.0018	...
FeCl ₃ · 6H ₂ O	0.9234	...	0.3719	0.2175	0.4340	0.0008	...
CaSO ₄	0.2585	0.0522	0.0619	0.1374
MgSO ₄
Total	4.2219	0.5304	0.4619	0.0650	0.1901	0.4446	0.1139	0.0083	0.1160	2.1370	0.0532	...

TABLE VIII. Salts used in the preparation of and elemental composition of nutritive solution E.

Salts used.	Concentration per litre. (Grams.)	Elemental composition.									
		Mg Gram.	Ca Gram.	Na Gram.	N Gram.	S Gram.	P Gram.	Fe Gram.	Cl Gram.	O Gram.	H Gram.
Ca(NO ₃) ₂ · 4H ₂ O	1.1131	...	0.3719	...	0.1901	0.6511	...
MgSO ₄ · 7H ₂ O	0.5000	0.0493	0.0631	0.3579	0.0286	...
CaH ₂ (PO ₃) ₂ · H ₂ O	0.4331	...	0.0736	0.1139	0.3645	0.0111
NaCl	0.1953	0.0650	0.1003
CaSO ₄ · 2H ₂ O	0.5000	...	0.1164	0.0931	0.2788	0.0117	...
FeCl ₃ · 6H ₂ O	0.0400	0.0083	0.0157	0.0142	0.0018	...
MgSO ₄	0.2485	0.0522	0.0689	0.1374	...
Total	3.0400	0.1012	0.4619	0.0650	0.1901	0.2271	0.0083	0.1160	1.7030	0.9533	...

Nutritive solution D was prepared as indicated in Table VII. It will be noticed that it possesses the same concentration per litre as solution C, but differs from it in containing more magnesium and sulphur, but less calcium, and a calcium-magnesium ratio of 4.55:1 instead of 10.9:1. Solution D differs from solution A in containing more magnesium, calcium, and sulphur, and in possessing a greater concentration. The differences between solutions D and A are more material than the differences between solutions A and C excepting in the matter of the calcium-magnesium ratio.

Nutritive solution E, as will be seen from a consideration of Table VIII, resembles closely solution D, but differs from it in containing less sulphur and a lower concentration—3.04 grm. per litre instead of 4.22 grm. Solution E contains the same concentration of salts as solution B, but a calcium-magnesium ratio of 4.55:1 instead of 10.9:1. In elemental composition it differs from solution B in containing more magnesium and sulphur, but less calcium.

TABLE IX. *Rate of growth of Blue stem Wheat plants in nutritive solutions A, D, and E.*

Growth measured after	Growth of plants in								
	Nutritive solution A.			Nutritive solution D.			Nutritive solution E.		
	Min.	Max.	Mean.	Min.	Max.	Mean.	Min.	Max.	Mean.
Days.	Cm.	Cm.	Cm.	Cm.	Cm.	Cm.	Cm.	Cm.	Cm.
3	7.0	9.0	8.5	8.0	10.0	9.2	6.0	8.5	7.4
4	10.5	13.0	11.8	12.0	13.5	12.6	7.5	11.5	9.8
5	14.0	16.0	14.9	14.0	17.0	15.8	10.0	14.5	12.7
6	15.5	18.0	16.5	16.0	19.5	18.0	12.0	16.5	14.4
7	16.0	20.0	17.6	18.0	21.0	19.0	14.0	17.0	15.8
9	18.0	24.0	22.1	21.0	25.0	23.5	15.0	18.5	16.4
13	25.0	28.0	26.6	27.0	32.0	28.3	16.0	24.0	20.2
18	33.5	40.5	36.5	34.0	41.0	37.5	18.0	24.5	21.1

The experiment was begun on October 3 and closed on October 24, that is, the plants were grown for twenty-one days as in Experiment 1. Again the full nutritive solutions behaved in a very similar manner and absence of potassium resulted in the same lack of vigour and gradual drying up of the foliar tissues. At the end of the experiment the plants in solutions A and D were healthy and growing vigorously, and possessed usually three stems and never less than two, and the roots were long, glistening, white, and well branched. The plants in solution E, on the other hand, were puny, showed no evidence of stooling, and their leaves were drying up from the tips, and irregularly in other parts of the blades, but these changes were not preceded by any visible chlorophyll decomposition. The root system of the plants was poorly developed, and the secondary roots were rarely 1 cm. long.

Measurements taken of the rate of growth of the plants permit one more accurately to visualize the effect of the various solutions on growth. The data obtained is given in Table IX. It will be noticed that already on

the third day the effect of absence of potassium was perceptible. The daily increment of growth of the plants in solution E during the first seven days was very nearly uniform, though distinctly less rapid than that of the plants in solutions A and D. After the seventh day, however, there is a marked falling off in the daily increment of growth, and on the eighteenth day stagnation is imminent; whereas in the plants in the full nutritive solutions growth continues uninterruptedly and sustainedly. It will also be noticed that the rate of growth of the plants in solution A is a little less

TABLE X. *Green and dry weights of Blue stem Wheat grown 21 days in nutritive solutions A, D, and E.*

Nutritive solution used.	No. of plants.	Total weight of plants.		Weight of tops.	Weight of roots.	Ratio of tops to roots.
		Green.	Dry.			
		Gram.	Gram.	Gram.	Gram.	Gram.
Nutritive Solution A	5	9.4606	0.8099	0.5219	0.1820	3.31
	5	14.1160	1.1238	0.8727	0.2511	3.47
	5	17.3300	1.2883	0.9459	0.3424	2.76
	5	12.4260	1.0415	0.7998	0.2417	3.31
	5	12.2380	0.9924	0.7573	0.2351	3.22
	5	14.1390	1.2062	0.9119	0.2943	3.09
Mean	1	1.6270	0.2154	0.1636	0.0518	3.16
Nutritive solution D	5	12.640	1.0783	0.8472	0.2311	3.66
	5	11.7530	1.0402	0.8319	0.2083	3.99
	5	14.4320	1.2863	1.0118	0.2746	3.68
	5	11.7410	0.9918	0.7575	0.2043	3.71
	5	13.5320	1.1734	0.9245	0.2459	3.72
	5	14.6910	1.2605	0.9732	0.2603	3.72
Mean	1	1.6138	0.2170	0.1782	0.0486	3.65
Nutritive solution E	5	1.2768	0.1606	0.1326	0.0280	4.73
	5	1.1294	0.1262	0.1276	0.0287	4.45
	5	1.4652	0.1764	0.1428	0.0336	4.25
	5	0.8076	0.1282	0.1289	0.0293	4.40
	5	1.2048	0.1568	0.1250	0.0318	3.93
	5	1.1698	0.1692	0.1328	0.0364	3.65
Mean	1	0.2351	0.0326	0.0263	0.0065	4.17

than that of the plants in solution D, though apparently this difference would not have persisted had the experiment continued longer.

The green and dry weights of the entire plants, the tops and roots, and the ratio of top growth to root growth at the conclusion of the experiment are given in Table X. The data given in the table show that the total dry weight of a plant grown in the absence of potassium was 0.0326 gram., that is, 19 mg. more than was obtained in Experiment 1—a wholly negligible quantity. In the case of the plants growing in solutions A and D, the mean dry weights of a plant were respectively 0.2154 gram. and 0.2170 gram.

The mean growth of a plant in solutions A and D was therefore better than that obtained in the full nutritive solutions used in Experiment 1. It will be remembered that in Experiment 1 the plants grown in solution C were the heavier; in Experiment 2 the plants grown in solution D are also the heavier. In Experiment 2 the mean dry weight of a plant grown in solution D was 9.9 per cent. greater than that of a plant grown in solution A; in Experiment 1 the difference in favour of solution C was 10.9 per cent. The behaviour of the plants grown in solutions C and D is therefore identical. We interpret this to mean that the change in concentration, not the salts used in its accomplishment, affected the rate of growth. A consideration of Table X also shows that root growth was relatively better in all solutions in Experiment 2 than in Experiment 1, the growth differences exhibited by the full nutritive solutions in the latter

TABLE XI. *Amount of and distribution of potassium in Blue stem Wheat plants grown 21 days in nutritive solutions A, D, and E.*

Nutritive solution used.	No. of plants.	Amount of potassium in			Potassium utilized per gramme of dry matter formed.		Ratio of potassium content of tops to roots.	Total potassium absorbed from the nutritive solution by one plant.
		Tops. Roots. Entire plants.			Tops. Roots.			
		Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.
Nutritive solution A	30	352.10	122.80	474.90	71.67	79.02	0.90	73.49
Nutritive solution D	30	395.30	109.30	505.20	74.01	74.82	0.99	74.18
Nutritive solution E	30	5.70	1.50	7.20	7.23	7.98	0.90	7.36

being again faithfully reproduced. The better relative root growth in the case of Experiment 2 is clearly not due, therefore, to the composition of solutions D and E, since it also occurred in solution A.

The potassium content of the plants and its relative distribution in tops and roots is indicated in Table XI. A study of the table shows that in all cases the total potassium absorbed per gramme of dry matter formed was heavier in Experiment 2 than in Experiment 1, but the distribution in roots and tops shows remarkably close agreement. In Experiment 1 the distribution ratio for the plants growing in solutions A and B is virtually identical; in Experiment 2 the distribution ratio for the plants growing in solutions A and E is identical. In Experiment 1 the potassium utilized per gramme of dry matter formed in the case of the plants growing in solution C is higher than that taken up by the plants growing in solutions A and B; similarly, in Experiment 2 the plants growing in solution D have a higher requirement than those growing in solutions A and E. The plants growing in solutions C and D have reacted similarly—further evidence that

TABLE XII. Salts used in the preparation of and elemental composition of nutritive solution F.

Salt used.	Concentration per litre.	Elemental composition.									
		K	Mg	Ca	Na	N	S	P	Fe	Cl	O
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	1.1131	0.2719	...	0.1901	0.6511
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.5000	...	0.0493	0.0631	0.3570
$\text{CaH}_2(\text{PO}_4)_2 \cdot \text{H}_2\text{O}$	0.4031	0.0736	0.0650	0.1139	0.2645
NaCl	0.1053	0.0083
$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	0.0400	0.0157
K_2SO_4	1.1820	0.5304	0.2175	...	0.0083	0.0157	0.4341
MgSO_4	0.2000	...	0.0404	0.0533	0.1063
Total	3.6635	0.5304	0.0897	0.3455	0.0650	0.1901	0.3359	0.1139	0.0083	0.1160	1.8272
											0.0415

TABLE XIII. Effect of potassium on rate of growth of Early pedigree Dent field Corn in sand culture.

Measurements taken after seedlings had come through sand.	Days.	Potassium present.			Potassium absent.		
		Min.	Max.	Mean.	Min.	Max.	Mean.
		Cm.	Cm.	Cm.	Cm.	Cm.	Cm.
8	8	11	15	13.8	11	15	13.3
13	13	24	28	26.5	18	24	20.4
15	15	28	33	31.0	21	28	23.5
18	18	34	42	37.1	24	31	27.3
20	20	38	47	41.7	26	32	28.7
22	22	41	49	45.5	28	35	30.1
27	27	51	64	56.9	30	40	35.0
30	30	55	76	61.9	33	43	37.1
35	35	61	86	68.1	34	43	37.7
41	41	71	86	76.7	34	43	37.7
46	46	77	95	83.4	34	43	37.7
55	55	85	105	91.8	34	43	37.7

slight changes in elemental composition are less important than changes in concentration.

Experiment 3. In this experiment Early pedigree Dent field Corn was grown in sand culture. The corn was carefully selected for size and only seeds weighing between 250 mg. and 350 mg. were used. The mean potassium content of a seed was determined by the analysis of three samples, each composed of several hundred seeds, and found to be 0.85 mg. The corn seed was therefore much richer in potassium than the wheat.

The sand employed for the cultures was a very fine and pure acid-washed silicon dioxide with a mean water saturation-point of 22.5 per cent. The pots used were made of glazed earthenware, and contained 1,400 grm. of the sand. Before planting the seed, water was added to the sand in sufficient quantity to give 60 per cent. of saturation. The water was added in the form of the appropriate nutritive solution and afterwards maintained by additions of distilled water as required. The full nutritive solution employed was solution F, the composition of which is given in Table XII. For the plants growing in the absence of potassium the same solution was employed, but without the addition of potassium. The full nutritive solution F contained 3.6635 grm. salts per litre, the less potassium solution F only 2.4815 grm.; but since wheat (see Table XX) yields identical results when grown in solutions free from potassium containing 3.6635 grm. salt and 2.4815 grm. salts per litre respectively, we assumed that the corn would behave similarly.

Eight pots were used for the full nutritive solution and eight for the nutritive solution less potassium, one plant being grown in each.

The experiment was begun on October 19, the seed, previously soaked sixteen hours in distilled water, being planted 1 cm. deep. Four days after planting the seedlings came through the sand, and after eight days measurements of rate of growth were taken periodically until the close of the experiment, the data obtained being given in Table XIII. A study of the table shows that potassium starvation gradually set in after the twelfth day from planting and that growth ceased after the thirty-fourth day in the absence of this element, while in the case of the plants growing in the full nutritive solution growth continued vigorously and uninterruptedly. The behaviour of the corn, therefore, whether growing in the presence or absence of potassium, resembles very closely that of wheat, the only difference worthy of note being that the symptoms of potassium starvation, which are in all points otherwise similar, appear more slowly, and extinction of growth is longer delayed.

After fifty-nine days the experiment was closed, and the plants from each pot weighed separately. The data obtained is given in Table XIV. It should be noted that the weights of the roots are approximate only, since it was neither possible to recover with certainty the entire root systems

nor to determine the dry weights directly. Furthermore, owing to the impossibility of separating the sand from the roots by lixiviation, the dry weights were obtained by difference as follows: The roots were dried and

TABLE XIV. *Green and dry weights of Early pedigree Dent field Corn grown in sand culture 39 days, both in the presence of and in the absence of potassium.*

Nature of culture.	No. of plants.	Dry weight.		Ratio of tops to roots.
		Entire plants.	Tops.	
Culture with potassium present.	1	4.7269	3.9580	0.7689
	1	6.0507	4.9792	1.0715
	1	4.9487	4.1896	0.7591
	1	5.7796	4.9048	0.8748
	1	4.6218	3.8597	0.7621
	1	5.5393	4.7709	0.7684
	1	6.2657	5.1724	1.0933
	1	5.3620	4.5222	0.8398
Mean	1	5.4118	4.5446	0.8672
Culture with potassium absent.	1	0.5352	0.4319	0.1034
	1	0.4616	0.3614	0.1002
	1	0.4272	0.3483	0.0789
	1	0.4124	0.3342	0.0782
	1	0.4221	0.3326	0.0895
	1	0.3169	0.2550	0.0619
	1	0.4636	0.3620	0.1016
	1	0.5360	0.4312	0.1048
Mean	1	0.4469	0.3571	0.0898

TABLE XV. *Amount of and distribution of potassium in Early pedigree Dent field Corn grown 39 days in sand culture free from and containing potassium.*

Name of culture.	No. of plants.	Amount of potassium in			Potassium utilized per gramme of dry matter formed.			Total potassium obtained from the panicle by one plant.	
		Tops.			Roots.			Total potassium utilized per gramme of dry matter formed.	Potassium obtained from the panicle by one plant.
		Mg.	Mg.	Mg.	Mg.	Mg.	Mg.		
Culture with potassium present.	8	2309.20	473.10	2784.30	63.52	68.47	0.92	64.31	347.19
Culture with potassium absent.	8	18.30	4.50	22.80	6.41	6.25	1.02	6.38	2.00

weighed in a platinum dish, ashed in a muffle furnace, and weighed again. The difference between these weights was assumed to be the weight of the roots.

A consideration of the data presented in Table XIV shows that the

growth of the plants in the full nutritive solution was twelve times larger than that of the plants grown in the absence of potassium.

The distribution of potassium in the plants is shown in Table XV. It will be noticed that the plants growing in the absence of potassium were able to obtain 2 mg. potassium from the substratum, and therefore grew under less favourable conditions for showing the effect of absence of this element than did the wheat in Experiments 1 and 2. Nevertheless, the ratio of potassium distribution in stems and roots per gramme of dry matter formed is the same whether the plants are grown in the presence or absence of potassium, results which agree with those obtained in the case of wheat. The figures for the amount of potassium absorbed per gramme of dry matter formed are also substantially the same as those given by wheat.

TABLE XVI. *Green and dry weights of Early pedigree Dent field Corn grown 32 days in water cultures free from and containing potassium.*

Nutritive solution used.	No. of Plants.	Total weight of plants.		Dry weight of		Ratio of tops to roots
		Green.	Dry.	Tops.	Roots.	
		Grm.	Grm.	Grm.	Grm.	
Nutritive solution F	3	35.305	3.9296	2.7996	1.1300	2.42
	3	40.920	4.2784	3.0881	1.1903	
	3	30.821	3.5357	2.5138	1.0119	
	3	28.592	3.3369	2.2564	1.0805	
Mean	1	11.306	1.2559	0.8882	0.3677	
Nutritive solution F, less potassium.	3	2.480	0.5067	0.3720	0.1356	2.75
	3	4.333	0.7630	0.5581	0.2047	
	3	6.769	0.9881	0.7191	0.2690	
	3	3.999	0.7014	0.5232	0.1792	
Mean	1	1.465	0.2467	0.1810	0.0657	

Experiment 4. In this experiment Early pedigree Dent field Corn was grown in water culture, solution F being used for the full nutritive solution, and without the addition of potassium for the solution less this element. Three corn plants were grown in each culture vessel, the jars used containing 1,600 c.c. solution. The seed was transferred to the jars when the primary root was about a half-centimetre long, and before the epicotyl had broken through the coleoptilum. At the end of thirty-two days the plants in the solution from which potassium was absent had ceased growing and were practically dead, while those in the full nutritive solution were perfectly healthy and growing vigorously. The experiment was, therefore, brought to a close and the plants weighed and analysed. The total green and dry weights of the plants and the ratio of top growth to root growth are given in Table XVI. The data presented show that the growth of the plants in the

absence of potassium was one-fifth that of the plants growing in the presence of this element, figures which are very similar to those obtained with wheat. The growth of the roots relatively to the tops was greater in the plants growing in the full nutritive solution than in those growing in the absence of potassium, a slight stunting of the root system being therefore produced in the absence of potassium, as in the case of wheat.

The distribution of the potassium in the plants is shown in Table XVII. It will be noticed that in the water culture experiment the corn growing in the absence of potassium obtained less than 1 mg. per plant of potassium from the solution. Nevertheless we still find that the relative distribution of the element per gramme of dry matter formed is practically the same whether the plant is grown in a full nutritive solution or in the potassium-free solution. The amount of potassium utilized per gramme of dry matter formed

TABLE XVII. *Amount of and distribution of potassium in Early pedigree Dent field Corn grown 32 days in water cultures free from and containing potassium.*

Nutritive solution used.	No. of plants.	Amount of potassium in			Potassium utilized per gramme of dry matter formed.		Ratio of potassium content of tops to roots.	Total potassium utilized from the nutritive solution per gramme of dry matter formed.	Potassium absorbed from the nutritive solution by one plant.
		Tops. Roots. Entire plants.			Tops. Roots.				
		Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.
Nutritive solution F	12	726.90	310.40	1037.30	68.21	70.35	0.96	68.8	85.59
Nutritive solution F, less potassium.	12	15.10	4.90	20.0	6.95	6.22	1.11	6.7	0.82

is 0.0688 gm. in the full nutritive solution, and 0.067 gm. in the solution less potassium, figures which agree with those obtained for wheat and corn (see Experiments 1, 2, and 3).

Experiment 5. In this experiment Japanese buckwheat was grown in water culture, solution F being used, as in Experiment 4, with and without the addition of potassium. An analysis of two samples of seeds containing respectively five hundred and eight hundred specimens showed that the mean potassium content of a single seed was 0.06 mg. The buckwheat was therefore much poorer in potassium than either the wheat or corn. Four plants were grown in each jar, and four jars were used for both the full nutritive and nutritive less potassium solutions. The seeds after two days in the germinator had grown sufficiently to be transferred to the culture solutions, the combined length of root and hypocotyl being about 2 cm. long, but the seed-coats had not been shed. Twenty-one days after the seedlings had been placed in culture the experiment was closed. At this

time the plants in the full nutritive solution were coming into flower and the inflorescence showed colour; the plants had four nodes and the ultimate leaf was unfolding. The plants growing in the absence of potassium, on the other hand, had stagnated since the twelfth day, and possessed but one short internode and a miniature leaf; the root system was also dwarfed, but otherwise resembled that of the plants growing in the full nutritive solution.

TABLE XVIII. *Green and dry weights of Japanese buckwheat grown in water cultures free from and containing potassium.*

Nutritive potassium used.	No. of plants.	Total weight of plants.		Dry weight of		Ratio of tops to roots.
		Green.	Dry.	Tops.	Roots.	
		Grm.	Grm.	Grm.	Grm.	
Nutritive solution F	4	8.052	0.8692	0.8181	0.0511	
	4	9.186	0.9250	0.8698	0.0552	
	4	10.605	1.0609	0.9941	0.0668	
	4	11.067	1.1388	1.0839	0.0549	
Mean	16	2.436	0.2496	0.2354	0.0142	16.5
Nutritive solution F, less potassium.	4	0.986	0.1350	0.1175	0.0125	
	4	0.834	0.1281	0.1193	0.0088	
	4	0.682	0.0869	0.0824	0.0045	
	4	0.678	0.1038	0.0950	0.0088	
Mean	16	0.199	0.0281	0.0259	0.0020	12.9

TABLE XIX. *Amount of and distribution of potassium in Japanese buckwheat grown in water culture free from and containing potassium.*

Nutritive solution used.	No. of plants.	Amount of potassium in			Potassium utilized per gramme of dry matter formed.		Ratio of potassium content of tops to roots.	Total potassium utilized per gramme of dry matter formed	Potassium absorbed from the nutritive solution by one plant.
		in			Potassium utilized per gramme of dry matter formed.				
		Tops.	Roots.	Entire plants.	Tops.	Roots.			
		Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.
Nutritive solution F	16	212.70	24.30	237.0	56.48	106.60	0.52	59.34	14.74
Nutritive solution F, less potassium	16	2.60	1.40	4.0	6.28	40.46	0.15	8.91	0.18

The green and dry weights of the plants are given in Table XVIII. A glance at the table shows that in the absence of potassium the dry weight of the buckwheat plants was 8.8 times less than that of the plants growing in the full nutritive solution. But it will be noticed that, contrary to what was found in the case of the wheat and corn, absence of potassium does not disturb the relative growth of tops and roots.

The distribution of potassium in the plants is shown in Table XIX.

It will be immediately noticed that in the buckwheat the roots are enormously richer in potassium than the tops. In the full nutritive solution the tops utilize only 0.53 grm. of potassium per gramme of dry matter formed against 1 grm. in the roots; in the case of the nutritive solution less potassium the difference is even more marked: the tops utilize 0.15 grm. of potassium for every gramme of dry matter formed, while the roots consume 1 grm. Japanese buckwheat behaves, therefore, in a manner wholly different from either wheat or corn as regards potassium utilization per gramme of dry matter formed in tops and roots, but similarly to both these plants when we consider the potassium consumed by the plant as a whole per gramme of dry matter formed. In the case of wheat and corn the consumption per gramme of dry matter formed in the full nutritive solution is 0.0727 and 0.0688 grm., in the nutritive solution less potassium 0.0063 and 0.0067, while in the case of Japanese buckwheat these figures become 0.0593 grm. and 0.0089 grm. respectively. In the absence of potassium, buckwheat is apparently unable to effect as economic a utilization of the element as either wheat or corn, and further evidence in favour of this view will be found on p. 223.

II. EFFECT OF DELAYED ADDITIONS OF POTASSIUM ON GROWTH.

In our experiments on the effect of delayed additions of potassium on growth we have used Blue stem Wheat, Early pedigree Dent field Corn, and Japanese buckwheat, all but one of the experiments being carried out in water culture. Some of the plants were grown in a full nutritive solution from the beginning, some in a solution free from potassium, while in the case of others potassium was usually added to the nutritive solution three, six, nine, and twelve days after the experiment was set up, though in one experiment with wheat the effect of additions delayed for longer periods was also tried. The nutritive solution (solution F) used was prepared as indicated in Table XII (see p. 201). Nutritive solution F has a concentration greater than that of solution A and less than that of solution C by almost exactly half the difference between the two. The calcium-magnesium ratio is 3.85:1, i.e. slightly lower than that of solution A. Solution F contains more magnesium, calcium, and sulphur than solution A, but otherwise resembles it more closely than it does any of the other nutritive solutions used. As will be seen from the table, the potassium in solution F was added as the sulphate instead of the nitrate as in solution A. This was done so as to permit the use of the solution for the cultures less potassium, and for those which were to receive potassium after the lapse of specified times. The concentration of the solution in the absence of potassium was 2.4815 grm. per litre instead of 3.6635 grm. The plants growing throughout an experiment in the absence of potassium were therefore in a weaker

solution than those growing in the full nutritive solution; and the plants growing partly in the absence of potassium were subjected to a change of concentration upon its addition from 2.4815 grm. per litre to 3.6635 grm. It seemed to us necessary to verify the fact that growing plants in the weaker concentration did not affect them adversely, and so in one experiment with wheat the concentration of the less potassium solution was increased to that of the full nutritive solution by the addition of calcium sulphate and magnesium sulphate in the ratio 3.85:1. The results obtained are indicated in Table XX, and show conclusively that the reduction in concentration used did not affect the growth of the plants. For convenience in working, a standard solution of potassium sulphate was made up, so that 25 c.c. contained the amount required per jar. In order to allow the addition of these 25 c.c., a like amount of nutritive solution was previously withdrawn

TABLE XX. *Effect of concentration of nutritive solution on growth of Blue stem Wheat plants in the absence of potassium.*

Concentration per litre.	No. of plants.	Total weight of plants.		Dry weight of		Ratio of tops to roots.
		Green.	Dry.	Tops.	Roots.	
Grm.		Grm.	Grm.	Grm.	Grm.	
3.6635	5	0.6570	0.1401	0.1120	0.0281	
	5	0.5803	0.1251	0.0969	0.0282	
Mean	1	0.1237	0.0265	0.0209	0.0056	3.73
2.4815	5	0.6461	0.1378	0.1086	0.0292	
	5	0.6393	0.1213	0.0962	0.0251	
Mean	1	0.1285	0.0259	0.0205	0.0054	3.79

and placed in small stoppered flasks until it could be returned. After the potassium had been added the jars were aerated to ensure thorough mixing.

Experiment 1. In this experiment Blue stem Wheat was used, and the plants were grown in solutions A and F, the latter both as a full nutritive solution and modified as required for the purpose of studying the effect of delayed additions of potassium. Three days after the seed had been placed in the germinator the seedlings had grown sufficiently to be transferred to the nutritive solutions. Five plants were grown in each jar, and there were two jars of plants in solutions A, F, and F less potassium, but four jars in each case for the study of the effect of delayed additions of potassium.

The experiment was set up on February 11 and allowed to run twenty-one days. At the close of the experiment the plants in the full nutritive solutions were healthy and growing vigorously, and had stooled; their root

systems were glistening, white, fibrous, and well branched. No observable difference could be detected between the plants growing in solution A and

TABLE XXI. *Effect of delayed additions of potassium on the growth of Blue stem Wheat plants in water culture.*

Nutritive solution used.	No. of plants.	Total weight of plants.		Dry weight of		Ratio of tops to roots.
		Green.	Dry.	Tops.	Roots.	
		Grm.	Grm.	Grm.	Grm.	
Solution A	5	5.3680	0.7406	0.5348	0.2056	
	5	8.4450	0.7299	0.5398	0.1901	
Mean	1	1.3813	0.1470	0.1075	0.0396	2.72
Solution F	5	6.9050	0.6613	0.4907	0.1706	
	5	8.6360	0.7795	0.6029	0.1766	
Mean	1	1.5541	0.1441	0.1094	0.0347	3.15
Solution F, less potassium.	5	2.3820	0.2826	0.2218	0.0608	
Potassium added after 3 days.	5	1.5330	0.2325	0.1815	0.0510	
	5	2.1650	0.3602	0.2702	0.0900	
	5	1.2650	0.2213	0.1731	0.0482	
Mean	1	0.4172	0.0548	0.0423	0.0125	3.38
Solution F, less potassium.	5	2.1270	0.2721	0.2160	0.0561	
Potassium added after 6 days.	5	1.9944	0.2752	0.2031	0.0721	
	5	0.9352	0.1605	0.1292	0.0313	
	5	1.4222	0.2014	0.1583	0.0431	
Mean	1	0.3239	0.0455	0.0353	0.0101	3.49
Solution F, less potassium.	5	0.7210	0.1705	0.1408	0.0297	
Potassium added after 9 days.	5	0.3632	0.1017	0.0793	0.0224	
	5	0.7500	0.1606	0.1270	0.0336	
	5	0.4087	0.1064	0.0845	0.0219	
Mean	1	0.1015	0.0246	0.0194	0.0052	3.73
Solution F, less potassium.	5 ¹	0.6591	0.1411	0.1133	0.0278	
Potassium added after 12 days.	5	0.9050	0.1510	0.1146	0.0364	
	5	0.4969	0.1169	0.0936	0.0233	
	5	0.6559	0.1516	0.1220	0.0296	
Mean	1	0.1358	0.0280	0.0212	0.0059	3.76
Solution F, less potassium.	1 ²	0.1261	0.0262	0.0207	0.0055	3.76

¹ In computing the mean, the data for these five plants were omitted, as an error was apparently made in taking the weight of either the tops or the roots.

² Mean of the data presented in Table XX.

those growing in solution F. The plants which received potassium after a lapse of three days were healthy, but much smaller than those in the full nutritive solutions, and their root system was poorly developed and dull white in colour, the secondary roots being short and stubby. Altogether, the tops appeared to have benefited by the addition of potassium more than the roots. The plants deprived of potassium for six days were markedly stunted and showed all the symptoms of potassium starvation. The plants growing in the absence of potassium for nine and twelve days respectively

TABLE XXII. *Effect of delayed additions of potassium on amount and distribution of potassium in Blue stem Wheat plants grown in water culture.*

Nutritive solution used.	No. of plants.	Amount of potassium in			Potassium utilized per gramme of dry matter formed.		Ratio of potassium content of tops to roots.	Total potassium utilized per gramme of dry matter formed.	Potassium absorbed from the nutritive solution by one plant.
		Tops. Roots. Entire plants.			Tops. Roots.				
		Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.
Solutions A and F, combined.	20	142.30	47.60	189.90	62.62	64.02	1.02	65.23	9.30
Solution F, less potassium. Potassium added after 3 days.	20	54.50	15.60	70.10	64.37	63.45	1.01	63.92	3.30
Solution F, less potassium. Potassium added after 6 days.	20	41.10	10.10	51.20	58.21	49.85	1.16	56.31	2.44
Solution F, less potassium. Potassium added after 9 days.	20	18.50	4.60	23.10	42.86	42.75	1.00	42.84	1.04
Solution F, less potassium. Potassium added after 12 days.	20	14.40	3.90	18.30	32.48	32.64	0.99	32.64	0.82
Solution F, less potassium.	20	3.20	0.80	4.00	7.72	6.95	1.11	7.63	0.06

had apparently failed to obtain any benefit from its addition: like the plants growing entirely in the absence of potassium, they were withering. The differences in the behaviour of the plants already well marked ocularily are more fully brought out when we study the data presented in Table XXI. It will be noticed that while the plants grown in solutions A and F show virtually the same total dry weight, they differ slightly from one another in the relative distribution of it. The root development in solution F is not quite as strong as in solution A.

The effect of delayed additions of potassium is seen to have very serious results even when the plants are only grown three days without this element present, and when grown nine and twelve days, respectively, without it not the slightest recovery follows its addition. It will also be noted that corresponding with the decrease in total dry matter formed there is a decrease in root growth. Potassium added to plants grown in its absence for nine and twelve days is entirely without effect, the ratio of top weight to root weight remaining similar to that of the plants grown throughout in a solution free from potassium—further proof that the plants had been unable to make any recovery.

But if the plants were unable to show any recovery from potassium starvation if the element were not furnished within six days, a consideration of Table XXII shows that they were, nevertheless, capable of absorbing potassium when it was offered. The relative distribution of potassium in tops and roots per gramme of dry matter formed is the same whether the plants have been grown in its absence or whether it has been offered to them at once or only after the lapse of a certain time. It will be noticed, however, that the amount of potassium utilized per gramme of dry matter formed is distinctly affected by the length of time the plants are permitted to obtain it. It will also be apparent from the table that the presence of potassium does not directly influence the amount of dry matter formed. In fact, a simple calculation will show the following relation to exist between potassium utilized and dry matter formed:

In the full nutritive solution there was formed by 1 gram. potassium				15.33 gram. dry matter.
(Mean of solutions A and F)				
When potassium was added after 3 days there was formed by 1 gram. potassium				15.62 gram. dry matter.
"	"	"	"	6 " " " by 1 gram. potassium
"	"	"	"	17.76 gram. dry matter.
"	"	"	"	9 " " " by 1 gram. potassium
"	"	"	"	23.34 gram. dry matter.
"	"	"	"	12 " " " by 1 gram. potassium
"	"	"	"	30.64 gram. dry matter.
When potassium was absent				" " by 1 gram. potassium
				131.06 gram. dry matter.

The above figures do not sustain the view that potassium is necessary to the synthesis and translocation of carbohydrates, and we have in fact noticed that Blue stem Wheat plants growing in the absence of potassium form and translocate starch as readily as plants growing in the presence of this element. Plants were grown in solution F plus potassium and in solution F less potassium in numbers sufficient to allow specimens being withdrawn from time to time over a period of three weeks. One jar of plants of each of the nutritive solutions was always taken for one examination, part of the plants being used in the test for starch, the remainder in the test for translocation. Eight days after the experiment was begun trinkled areas—that is, the first symptoms of potassium starvation—began to

appear on the leaves of the plants growing in the absence of potassium, and a test for starch and translocation was made. Early in the afternoon, the day being bright and sunny, leaves were gathered, boiled in water for a few minutes, washed in several changes of alcohol until white, transferred to a flat-bottomed white porcelain dish, washed with water to remove the alcohol, and then floated in Gram's iodine solution. The leaves from the plants growing in the nutritive solution less potassium gave a starch reaction of the same intensity as those from the plants growing in the presence of potassium. The plants from which the leaves had been taken were transferred to a dark chamber for forty-two hours. The remaining leaves were then tested for starch, but none was found. The experiments just described were several times repeated; starch was always found present in the leaves and stems, absence of potassium being without effect on the intensity of the reaction obtained, and tests on the rate of translocation, the plants being kept in the dark for various lengths of time from eighteen to seventy-two hours, gave no evidence at all that absence of potassium interfered with this function.

At the end of twenty-one days the plants growing in the absence of potassium had ceased growing and were withering. Nevertheless, the green portions of the leaves still assimilated and the starch was translocated as readily as from the leaves of the plants growing in the full nutritive solution.

Experiment 2. In this experiment Blue stem Wheat was grown to maturity in sand culture, 1,400 grm. sand being used per pot with a water content of 60 per cent. of saturation, the water required being partly added in the form of nutritive solution F, less potassium. The wheat, after washing in distilled water for sixteen hours, was planted 1 cm. deep on April 23, enough seed being supposedly planted to allow ten plants per pot, but the stand desired was not obtained in all cases. Seven days after planting the seedlings had come through the sand about 5 cm., and the experiment proper was begun. At this time the eight pots that had been planted were numbered consecutively and potassium was added as per the following schedule: Pot No. 1 received immediately the requisite complement of potassium sulphate to make a full nutritive pabulum; pot No. 2 received the requisite complement of potassium three days later; pot No. 3, six days later; pot No. 4, nine days later; pot No. 5, twelve days later; pot No. 6, seventeen days later; pot No. 7, twenty-two days later; while pot No. 8 never received any potassium. The experiment was discontinued eighty-seven days after potassium was added to pot No. 1, or ninety-four days from the day the seeds were planted.

The plants were measured on the day the experiment was begun, and periodically thereafter for sixty-three days. The figures obtained are indicated in Table XXIII. A consideration of the data presented in the

table shows that delayed additions of potassium had eventually an effect on rate of growth, there being after a lapse of time an increase in the daily increment. When the addition of potassium was withheld three days the increase in rate of growth only became manifest after eighteen days; when the potassium was not supplied until the sixth day, twenty-three days elapsed before any change in rate of growth occurred; when potassium was withheld nine and twelve days respectively, it was thirty-one days before the growth-rate improved; when the potassium was withheld seventeen days, no improvement was visible for thirty-six days; and when potassium was not added until the twenty-second day, forty-eight days elapsed before any beneficial effect on rate of growth could be observed. It will be noticed that after sixty-three days the full nutritive milieu and the milieus which received potassium after three, six, nine, twelve, and seventeen days had all

TABLE XXIII. *Effect of delayed additions of potassium on rate of growth of Blue stem Wheat plants in sand culture.*

Measure- ments taken after planting of seed.	Potassium present from the beginning.	Potassium added after 3 days.	Potassium added after 6 days.	Potassium added after 9 days.	Potassium added after 12 days.	Potassium added after 17 days.	Potassium added after 22 days.	Potassium absent.
Days.	Cm.	Cm.	Cm.	Cm.	Cm.	Cm.	Cm.	Cm.
7	5.0	5.4	4.5	4.8	4.6	6.6	5.1	5.7
10	6.4	6.6	6.0	7.2	6.6	8.1	6.8	7.1
13	7.3	7.1	6.2	8.6	8.4	10.6	8.0	8.4
16	9.1	8.5	7.6	9.6	10.2	12.3	9.6	10.3
20	11.3	9.5	8.3	10.3	10.7	12.7	10.0	10.7
25	13.1	11.8	10.0	10.7	11.5	13.2	11.2	11.2
30	14.9	13.6	12.1	11.9	12.7	14.0	12.1	11.8
38	16.1	17.2	15.5	15.0	14.5	14.4	12.2	11.9
43	20.3	20.6	19.1	18.4	17.9	17.3	12.4	12.1
49	23.7	24.6	22.4	22.3	20.2	20.9	12.8	12.8
55	26.8	29.3	26.3	27.4	25.0	26.6	16.6	14.1
63	30.1	33.4	28.2	31.4	27.8	32.3	21.2	16.1
70	31.4	35.2	29.7	33.7	30.3	34.0	26.2	18.0

produced plants that were apparently equally vigorous and healthy; but that a gradual and progressive falling off in rate of growth occurred when the addition of potassium was not made for nine, twelve, seventeen, and twenty-two days, though recovery is obvious even when the element is withheld twenty-two days.

When the experiment was discontinued all the plants capable of so doing had come to maturity, and the others were drying up. Most of the plants had formed heads, except those grown in the absence of potassium, but the seed was quite immature in all the plants with the exception of those growing in pots No. 1 and No. 2. One effect of absence of potassium during the early stages of growth is then to delay maturation, though, as will be seen from a consideration of the data given in Table XXIV, the production of dry matter is not affected.

A study of the figures given in the table also shows that when the additions of potassium are delayed more than twelve days the effects of

starvation are not overcome, and are more marked than the figures on rate of growth given in Table XXIII, taken in themselves, would lead one to expect. The data presented in Table XXIV would also lead one to expect that the total amount of potassium utilized per gramme of dry matter formed would be approximately the same for plants growing in the presence of potassium from the beginning or suffering from a period of starvation up to and including twelve days. The data presented in Table XXV confirms this expectation, and shows that while the amount of potassium utilized per gramme of dry matter formed is slightly irregular, there is no indication of a relation such as was found in the water-culture experiment (see Table XXII) between the potassium requirement and delayed additions of the element. In the present instance, however, the period of growth was of sufficient duration for the plants longest starved to show well-marked evidences of recovery, which was not the case in Experiment 1.

TABLE XXIV. *Effect of delayed additions of potassium on the dry weight produced by Blue stem Wheat plants.*

No. of pot.	Method of treatment.	No. of plants.	No. of plants with heads.	Total dry weight of plants.	Mean dry weight of one plant.
				Grm.	Grm.
1	Potassium present from the beginning.	9	5	3.2044	0.5341
2	Potassium added after 3 days.	7	7	3.9672	0.5668
3	" " " 6 "	7	6	4.4738	0.6391
4	" " " 9 "	8	7	4.8323	0.6040
5	" " " 12 "	7	7	4.0373	0.5768
6	" " " 17 "	10	9	3.6162	0.3626
7	" " " 22 "	10	7	2.5014	0.2531
8	" absent	10	1	0.4766	0.0476

In Experiment 1 the wheat was grown twenty-one days in the nutritive solutions, in the experiment under consideration eighty-three days elapsed between the setting up and taking down: in other words, Experiment 2 was run almost four times longer than Experiment 1. If we assume that the daily increment of potassium absorption of the plants growing in the absence of potassium was the same in both experiments, then in Experiment 2 the plants should contain four times the amount of this element present in the plants of Experiment 1. The amounts actually were 0.34 mg. per plant for Experiment 2, and 0.09 mg. per plant for Experiment 1. Obviously, therefore, the differences in potassium requirement shown by plants subjected to delayed additions in Experiments 1 and 2 in a measure represent recovery effected. We say in a measure represent the recovery effected, because the disturbing effect on the plant metabolism that exists during the interval of starvation and in the interval of time before the establishment of equilibrium following the addition of potassium retards

by just so much the normal life cycle. We found, it will be remembered, the plants of wheat at different stages of maturity when Experiment 2 was concluded. The complete life cycle would not have occurred in the potassium-starved plants, but would have reached completion in those plants that were supplied with the element.

Experiment 3. Early pedigree Dent field Corn was grown in water culture in this experiment, solution F being used. The plants were grown in the full nutritive solution, in the nutritive solution less potassium, and in the nutritive solution, to which potassium was only added after three, six, nine, and twelve days, four jars containing three plants being used in each case. The experiment was begun on April 5, the seedlings placed in the

TABLE XXV. *Effect of delayed additions of potassium on the amount of potassium in Blue stem Wheat plants grown in sand culture.*

Method of treatment.	No. of plants.	Total amount of potassium.	Amount of potassium in one plant.	Total potassium utilized per gramme of dry matter formed.	Potassium absorbed from the pabulum by one plant.
				Mg.	Mg.
Potassium present from the beginning.	6	264.5	44.05	82.48	29.94
Potassium added after 3 days.	7	285.6	40.8	72.00	40.69
Potassium added after 6 days.	7	264.5	43.5	68.27	43.39
Potassium added after 9 days.	8	342.0	42.7	70.76	42.59
Potassium added after 12 days.	7	215.0	45.0	78.02	44.89
Potassium added after 15 days.	10	261.1	26.11	72.01	26.00
Potassium added after 22 days.	10	162.1	16.20	64.82	16.90
Potassium absent.	10	4.5	0.45	9.45	0.34

jars then having roots about 0.7 cm. long, but the epicotyl was still enclosed in the coleoptilum. At the end of thirty-two days the experiment was brought to a close, as the plants growing in the absence of potassium had not only ceased growing but had practically dried up, exhibiting all the symptoms of potassium starvation. The plants growing in the full nutritive solution, as well as those which had received potassium after three and six days, were, on the other hand, all healthy and growing normally. The plants deprived of potassium for nine days showed symptoms of starvation, but were growing. The plants deprived of potassium for twelve days, while still growing, had their lowest leaves completely withered, and the others with tips and edges partly dried. The general effect produced on dry weight and on the ratios of top weight to root weight of the plants in the

several cultures is shown in Table XXVI. It will be noticed that the ratio of top weight to root weight is not affected by potassium starvation, but that serious loss of weight of the plant as a whole results when potassium is withheld for a longer period than three days.

TABLE XXVI. *Effect of delayed additions of potassium on the growth of Early pedigree Dent field Corn in water culture.*

Nutritive solution used.	No. of plants.	Total weight of plants.		Weight of		Ratio of tops to roots.
		Green.	Dry.	Tops.	Roots.	
		Grm.	Grm.	Grm.	Grm.	
Solution F. Potassium present from beginning.	3	35.305	4.2784	2.7996	1.1300	2.42
	3	40.950	3.9296	3.0881	1.1903	
	3	30.821	3.5257	2.5138	1.0119	
	3	28.592	3.3369	2.2564	1.0805	
Mean	1	11.306	1.2559	0.8882	0.3677	
Solution F, less potassium. Potassium added after 3 days.	3	37.957	4.3846	2.8224	1.0561	2.45
	3	33.737	4.0899	2.6418	1.1078	
	3	32.863	3.7885	3.1555	1.2291	
	3	36.586	3.7496	2.8255	1.2644	
Mean	1	11.762	1.3419	0.9538	0.3881	
Solution F, less potassium. Potassium added after 6 days.	3	35.221	3.6672	2.5523	1.1149	2.81
	3	28.940	3.0979	2.3061	0.7918	
	3	35.595	3.5670	2.9291	0.9379	
	3	32.179	3.3678	2.5317	0.8361	
Mean	1	10.995	1.1666	0.8599	0.3067	
Solution F, less potassium. Potassium added after 9 days.	3	22.909	2.9755	2.2008	0.7747	2.56
	3	24.337	2.7349	1.8901	0.8448	
	3	23.835	2.8934	2.0492	0.8442	
	3	26.051	2.7764	2.0442	0.7322	
Mean	1	8.094	0.9482	0.6820	0.2663	
Solution F, less potassium. Potassium added after 12 days.	3	17.060	2.0709	1.5020	0.5689	2.84
	3	10.644	1.2989	0.9587	0.3102	
	3	14.938	1.5788	1.1697	0.4091	
	3	19.266	1.3177	1.7147	0.6030	
Mean	1	5.234	0.6055	0.4479	0.1576	
Solution F. Potassium absent.	3	2.480	0.5067	0.3720	0.1356	2.75
	3	4.333	0.7630	0.5583	0.2047	
	3	6.769	0.9881	0.7191	0.2690	
	3	3.999	0.7024	0.5232	0.1792	
Mean	1	1.465	0.2467	0.1810	0.0657	

The distribution of potassium in the plants is shown in Table XXVII. It will be noticed that the relative distribution of potassium in roots and tops is throughout the same, thus agreeing with all previous experiments

TABLE XXVII. *Effect of delayed additions of potassium on the amount of potassium in Early pedigree Dent field Corn grown in water culture.*

Nutritive solution used.	No. of plants.	Amount of potassium in			Potassium utilized per gramme of dry matter formed.		Ratio of potassium content of tops to matter roots.	Total potassium utilized per gramme dry matter formed.	Potassium absorbed from the nutritive solution by one plant.
		Tops.	Roots.	Entire plants.	Tops.	Roots.			
		Mg.	Mg.	Mg.	Mg.	Mg.		Mg.	Mg.
Solution F.	12	726.9	310.4	1037.3	68.21	70.35	0.96	68.8	85.59
Solution F, less potassium. Potassium added after 3 days.	12	794.8	320.2	1115.0	69.45	68.76	1.01	69.2	92.06
Solution F, less potassium. Potassium added after 6 days.	12	576.9	210.8	787.7	55.91	57.26	0.97	56.2	64.79
Solution F, less potassium. Potassium added after 9 days.	12	359.0	144.2	503.2	43.87	45.12	0.97	44.2	41.08
Solution F, less potassium. Potassium added after 12 days.	12	210.5	73.2	283.7	39.16	38.72	1.01	39.0	22.79
Solution F. Potassium absent.	12	15.1	4.9	20.0	6.95	6.22	1.11	6.7	0.82

with corn and wheat; that the potassium utilized per gramme of dry matter formed is slightly higher when this element is only added after three days, and then decreases rapidly, the figures obtained agreeing remarkably with those given in previous experiments with corn and wheat. Again, as in the case of wheat (see p. 211), there is no direct relation shown between potassium absorbed and dry matter formed, as the following figures indicate:

In the full nutritive solution there was formed by 1 gram. potassium	14.52 gram. dry matter.
When potassium was added after 3 days there was formed by 1 gram. potassium	14.46 gram. dry matter.
" " " " 6 " " " " by 1 gram. potassium	17.77 gram. dry matter.
" " " " 9 " " " " by 1 gram. potassium	22.67 gram. dry matter.
" " " " 12 " " " " by 1 gram. potassium	25.64 gram. dry matter.
When potassium was absent " " " " by 1 gram. potassium	149.25 gram. dry matter.

When potassium was not added to the nutritive solution until the

third day, wheat, it will be remembered, lost 62 per cent. of its weight, but corn is not deleteriously affected at all. In fact, the mean weight of a plant of corn grown in the absence of potassium for three days is about 7 per cent. greater than that of a plant grown *ab initio* in the presence of potassium. The difference in behaviour of corn and wheat is significant. To what is it due? In the case of wheat the plants growing in the full nutritive solution used 1 gram. potassium for every 15.33 gram. of dry matter formed, while those growing in the nutritive solution to which potassium was added after three days produced 15.65 gram. of dry matter for every gramme of potassium absorbed. The figures for corn are: Plants growing in the full nutritive solution produced 14.52 gram. of dry matter per gramme of potassium, and those growing in the solution to which potassium was added after three days formed 14.46 of gram. dry matter per gramme of potassium absorbed. The relation of potassium to dry matter formed is very similar in both wheat and corn, the only difference being that a delayed addition curtails growth in the former and not in the latter. We interpret this to mean that some substance necessary for growth and to the formation of which potassium is essential was present in larger quantity in the corn than in the wheat.

Potassium is known to be present in relatively greater abundance in young organs.¹ Weevers² believed that potassium functions in the regulation of turgidity and in the synthesis and metabolism of proteids. Lüpke held the view that potassium does not exercise a limited and special function, but plays a rôle in metabolism more general in nature, and, like nitrogen, phosphorus, sulphur, &c., is essential to the growth of each individual cell. Loew³ considered potassium essential in the synthesis of carbohydrates, fat, and proteins, its rôle in the reactions being that of a condensing agent. He also noted a fact, not without importance, that in seeds there is a closer relation between potassium and protein than between potassium and starch. Basing his calculations on the analyses of Wolf ('Aschen-Analysen', vol. i), he found that the mean ratio of potassium to protein in the Gramineae was 1 : 17 and in the Leguminosae 1 : 23.

With the above opinions the results we have obtained do not conflict, with the exception of the relation to carbohydrate synthesis, which it does not seem to us the data presented in this paper support.

Experiment 4. Japanese buckwheat was used in this experiment, and the plants were grown in water culture, solution F being used as the nutritive medium. The plants were grown in the full nutritive solution, in

¹ Pfeffer, W.: *Physiology of Plants*, English trans., vol. i, p. 430, and literature there cited.

² Weevers, T.: *Untersuchungen über die Lokalisation und Funktion des Kaliums in der Pflanze*. Rec. Trav. Bot. Néerland., viii, 289-332, 1911.

³ Lüpke, R.: *Über die Bedeutung des Kaliums in der Pflanze*. Landw. Jahrb., xvi, 887-915, 1888.

⁴ Loew, O.: *The Physiological Role of Mineral Nutrients*. Div. Veg. Phys. and Path., U.S. Dept. Agr., Bull. xviii, p. 26 et seq., 1899.

solution F less potassium, and in solution F to which potassium was only added after three, six, nine, and twelve days. Four jars containing four plants each were used in every instance, the seedlings being placed in the jars when the combined length of root and hypocotyl was 2 cm. The experiment was begun on April 6 and was discontinued after twenty-two days. Growth measurements were taken periodically and the data obtained are shown in Table XXVIII. A study of the table shows that the rate of growth of the plants in the full nutritive solution was during the first three days somewhat greater than that of the plants growing in the absence of potassium, and continued very uniformly throughout the remaining eighteen days. After the third day the growth of the plants in the solution less potassium falls off, while that of the plants growing in the solution which received its potassium complement on the third day continues unimpaired, and at the close of the experiment is very nearly equal to that of the plants growing in the full nutritive solution. After six days the growth of the plants in the solution less potassium virtually ceases, but an immediate response is shown whenever potassium is added, even when the addition is delayed until the twelfth day.

After nine days' growth, the plants in the full nutritive solution had long stout hypocotyls, broad cotyledons, and two leaves unfolded; the plants growing in the solution to which potassium was only added after three days were somewhat smaller in all their parts than the plants growing in the full nutritive solution; the plants growing in the solution to which potassium was added on the sixth day were considerably smaller than the plants growing in the full nutritive solution, and the cotyledons showed slight marginal chlorosis which had in some cases a tendency to work inwards towards the veins: in the case of the plants growing in the potassium-free solution, one-fourth showed marked intraveinal chlorosis with a tendency towards albinism and drying margins, while the remaining plants showed less-pronounced symptoms of disease.

After fifteen days the plants growing in the full nutritive solution and in the solution to which potassium was added after three days were much alike: two pairs of leaves had expanded and the flower buds were developing. The plants growing in the solution to which potassium was supplied after six days were undersized; they had two pairs of leaves unfolded, but only two out of sixteen plants possessed flower buds. The plants growing in the solution to which potassium was not added until the ninth day showed still greater loss of stature; they had two pairs of leaves unfolded, but flower buds had not formed. The plants growing in the solution to which potassium was added after twelve days had made no response, and, like these growing in its entire absence, showed marked chlorosis and marginal drying of the leaves. After twenty-one days the plants growing in the full nutritive solution and in the solution to which potassium was

TABLE XXVIII. Effect of delayed additions of potassium on the rate of growth of Japanese buckwheat in water culture.

Measurement taken after Days.	Solution G, containing potassium from the beginning.			Solution F, less potassium, added after 3 days.			Solution F, less potassium, added after 6 days.			Solution F, less potassium, added after 9 days.			Solution F, less potassium, added after 12 days.			Solution F, Potassium absent.		
	Min.	Max.	Mean.	Min.	Max.	Mean.	Min.	Max.	Mean.	Min.	Max.	Mean.	Min.	Max.	Mean.	Min.	Max.	Mean.
3	2.5	5.5	4.3	3.0	5.5	4.1	3.0	5.0	4.1	3.0	5.0	4.1	3.0	5.5	4.1	2.0	5.5	4.1
6	6.0	11.0	8.6	5.0	9.0	7.4	5.0	7.0	5.8	5.0	7.0	6.1	3.0	7.0	5.8	4.0	8.0	5.9
9	8.0	12.0	10.1	7.0	10.0	9.2	5.0	8.0	6.7	5.0	7.0	6.1	4.0	8.0	5.9	4.0	9.0	6.0
12	10.0	15.0	12.8	9.0	14.0	11.8	7.0	9.0	8.0	6.0	9.0	6.9	4.0	8.0	6.4	5.0	9.0	6.5
15	14.0	19.0	16.4	12.0	17.0	14.9	7.0	12.0	9.2	6.0	9.0	7.3	4.0	9.0	6.6	5.0	9.0	6.5
18	16.0	27.0	22.0	17.0	25.0	21.7	9.0	16.0	12.9	6.0	14.0	8.9	4.0	10.0	7.4	5.0	9.0	6.5
21	21.0	32.0	26.4	20.0	31.0	26.1	9.0	23.0	15.9	6.0	15.0	11.0	4.0	13.0	8.7	5.0	9.0	6.5

added after three days were very similar: four nodes had developed, the ultimate leaves were unfolding, and the flowers were showing colour. The plants growing in the solution to which potassium was added after six days were smaller than those growing in the full nutritive solution and their inflorescence was less highly developed, otherwise the only symptoms of

TABLE XXIX. *Effect of delayed additions of potassium on the weight of Japanese buckwheat plants grown in water culture.*

Nutritive solution used.	No. of plants.	Total weight of plants.		Dry weight of		Ratio of tops to roots.
		Green.	Dry.	Tops.	Roots.	
		Grm.	Grm.	Grm.	Grm.	
Solution F. Potassium present from the beginning.	4	8.052	0.8692	0.8181	0.0511	16.5
	4	9.186	0.9250	0.8698	0.0552	
	4	10.665	1.0609	0.9941	0.0668	
	4	11.067	1.1388	1.0839	0.0549	
Mean	1	2.436	0.2496	0.2354	0.0142	
Solution F. less potassium. Potassium added after 3 days.	4	10.213	0.9306	0.8771	0.0535	15.1
	4	7.696	0.8188	0.7667	0.0521	
	4	9.285	0.9255	0.8637	0.0618	
	4	10.231	0.9798	0.9199	0.0599	
Mean	1	2.339	0.2284	0.2142	0.0142	
Solution F. less potassium. Potassium added after 6 days.	4	5.653	0.5179	0.4781	0.0398	11.5
	4	5.475	0.4451	0.4053	0.0398	
	4	6.006	0.5686	0.5252	0.0434	
	4	5.857	0.5960	0.5658	0.0302	
Mean	1	1.312	0.1205	0.1109	0.0096	
Solution F. less potassium. Potassium added after 9 days.	4	2.560	0.2555	0.2350	0.0205	10.8
	4	3.787	0.3446	0.3177	0.0269	
	4	3.085	0.3219	0.2991	0.0228	
	4	2.270	0.2331	0.2054	0.0277	
Mean	1	0.731	0.0722	0.0661	0.0061	
Solution F. less potassium. Potassium added after 12 days.	4	1.771	0.1736	0.1617	0.0119	11.1
	4	1.455	0.1461	0.1335	0.0126	
	4	1.784	0.1769	0.1662	0.0104	
	4	2.545	0.2411	0.2146	0.0265	
Mean	1	0.472	0.0461	0.0425	0.0038	
Solution F. Potassium absent.	4	0.986	0.1300	0.1175	0.0125	12.9
	4	0.834	0.1281	0.1193	0.0088	
	4	0.682	0.0869	0.0824	0.0045	
	4	0.678	0.1038	0.0950	0.0088	
Mean	1	0.199	0.0281	0.0259	0.0020	

potassium starvation remaining were slight marginal chlorosis and drying of the cotyledons. The plants growing in the solution to which potassium was added after nine days were undersized and had poorly developed inflorescences, but the only symptoms of potassium starvation were on the cotyledons, which were affected with marginal chlorosis. The plants growing in the solution to which potassium was added on the twelfth day were much dwarfed, only two nodes having developed and the cotyledons were chlorotic and possessed more or less dry revolute margins, but only in a few cases were the margins of the leaves chlorotic. The plants growing in the potassium-free solution possessed one short node and a miniature

TABLE XXX. *Effect of delayed additions of potassium on the amount of potassium present in Japanese buckwheat grown in water culture.*

Nutritive solution used.	No. of plants.	Amount of potassium in			Potassium utilized per gramme of dry matter formed.		Ratio of potassium utilized per gramme of dry matter formed to		Total potassium absorbed from the nutritive solution by one plant.	
							tops to roots.			
		Tops.	Roots.	Entire plants.	Tops.	Roots.	Mg.	Mg.	Mg.	Mg.
Solution F.	16	212.7	24.3	237.0	56.48	106.60	0.52	59.34	14.71	
Solution F, less potassium. Potassium added after 3 days.	16	204.8	20.9	225.7	59.75	91.95	0.64	61.76	14.04	
Solution F, less potassium. Potassium added after 6 days.	16	99.1	13.3	112.4	35.82	86.81	0.64	58.31	6.66	
Solution F, less potassium. Potassium added after 9 days.	16	54.4	7.7	62.1	51.46	78.65	0.65	53.76	3.82	
Solution F, less potassium. Potassium added after 12 days.	16	32.9	4.7	37.6	48.65	76.76	0.63	50.97	2.29	
Solution F. Potassium absent.	16	2.6	1.1	4.0	6.28	10.16	0.15	8.91	0.18	

leaf. The root systems of all the plants except those growing in the full nutritive solution showed a dwarfing which was the more marked the longer potassium was withheld.

The differences just described are well brought out in the data presented in Table XXIX. It will be noticed that the plants growing in the full nutritive solution and in the solution to which potassium was added after three days were able to grow relatively better tops than the plants growing in the other solutions, all of which behave in a very similar manner. Recovery, which was detected in the growth measurements, is not so

promptly exhibited, it will be noticed, in the ratios of top weight to root weight. The tops recover more promptly than do the roots.

The distribution of potassium in the plants is shown in Table XXX. It will be noticed that the relative amount of potassium utilized by the tops and roots per gramme of dry matter formed is the same whether the potassium was added to the nutritive solution at the beginning of the experiment or only after twelve days, but that absence of potassium for the entire period of growth causes a marked change in the ratio to the advantage of the roots. In the absence of potassium, therefore, Japanese buckwheat does not behave like wheat and corn. It should, however, be noticed that in the case of wheat and corn the potassium requirements of the tops and roots per gramme of dry matter formed is substantially the same, whereas in the case of Japanese buckwheat the requirement of the roots is very nearly twice that of the tops. In other words, the roots of buckwheat require for normal development per gramme of dry matter formed twice as much potassium as the tops. In starvation, therefore, we would expect the roots of this plant to effect a less economical use of potassium than the tops, and the differences existing in the normal plant to be consequently exaggerated in the starved plant, but that the normal ratio would be established upon the addition of potassium to the nutritive solution. This is exactly what happens, as the figures in Table XXXI will show.

TABLE XXXI. *Effect of potassium starvation on the amount of dry matter produced per gramme of potassium utilized in tops and roots of Japanese buckwheat.*

Nutritive solution used.	Dry matter formed per gramme of potassium utilized.	
	Tops.	Roots.
	Grm.	Grm.
Full nutritive solution	17.7	9.4
Nutritive solution plus potassium after 3 days	16.7	10.9
" " " 6 "	17.8	11.5
" " " 9 "	19.4	12.7
" " " 12 "	20.6	13.0
Nutritive solution less potassium	15.2	24.7

A study of Table XXX will further show that, as was found in the case of corn, the potassium utilized per gramme of dry matter formed is slightly higher when the element is only added to the nutritive solution on the third day. In the case of corn a slight increase in dry weight was at the same time recorded, but in the case of Japanese buckwheat a loss of weight was obtained of about the same magnitude. This behaviour indicates that the potassium content of the seed does not primarily determine the length of time potassium may be withheld without causing loss of weight. A plant grown from Blue stem Wheat seed, which contains 0.11 mg. of potassium, was unable to recover when this element was withheld three days, but a plant of

Japanese buckwheat obtained from seed containing 0.06 mg. suffered a corresponding loss in weight only when potassium was withheld six days, and an Early Dent Corn seed containing 0.85 mg. of potassium gave a plant that only showed similar stunting when potassium was withheld twelve days. If the potassium content of the seed were the determining factor, then starvation symptoms should have appeared in the following order—buckwheat, wheat, corn. It should, however, be remembered that wheat and corn are physiologically alike in their potassium requirement, while buckwheat does not resemble these plants at all in its potassium requirement. Potassium-starved wheat and corn are able to effect a more economical use of potassium than buckwheat, though they do not recover from potassium starvation as readily. It will be remembered that as potassium starvation was prolonged in wheat and corn, the dry matter produced per gramme of potassium utilized increased; in the case of buckwheat, however, the potassium requirement changes little, as the following figures show:

In the full nutritive solution there was formed by 1 grm. potassium						16.86 grm. dry matter.
When potassium was added after 3 days there was formed by 1 grm. potassium						16.19 grm. dry matter.
"	"	"	"	6	"	by 1 grm. potassium
"	"	"	"	"	"	17.16 grm. dry matter.
"	"	"	"	9	"	by 1 grm. potassium
"	"	"	"	"	"	18.60 grm. dry matter.
"	"	"	"	12	"	by 1 grm. potassium
"	"	"	"	"	"	19.61 grm. dry matter.
When potassium was absent						by 1 grm. potassium
						112.23 grm. dry matter.

In the case of buckwheat, therefore, we have a plant which, while unable to gain in weight as readily as wheat and corn in the absence of potassium, is nevertheless better able to recover from the effects of potassium starvation. Nobbe, Schroeder, and Erdemann¹ noticed that buckwheat was practically unable to synthesize starch in the absence of potassium, but that this function was resumed when potassium was added to the nutritive solution, and we have shown that starved plants renew growth when potassium is added to the nutritive solution. On the other hand, we have also shown that in the absence of potassium wheat continues to synthesize starch, but does not recover so readily from potassium starvation. Nevertheless, the rôle of potassium in metabolism must be the same in wheat, corn, and buckwheat; and of the rôles assigned to potassium preponderance should not be given, it seems to us, to synthesis of starch, though it must be recognized that a disturbance in the normal functioning of any metabolic or catabolic process usually has a more or less sensible effect on all life-processes of the cell, but the magnitude of the disturbance and the exact secondary reactions that will ensue need not of necessity be invariably the same.

¹ Loc. cit. ante.

SUMMARY.

1. The reserve supply of potassium in the seed is not sufficient to maintain normal growth except for a very short period of time.
2. Symptoms of potassium starvation appear early in the life of a plant, and are characterized by a dwarfing of the axis and progressive death of the foliage, the older leaves succumbing first.
3. The potassium absorbed per gramme of dry matter formed is higher in plants growing in the presence of potassium than in plants growing in its absence.
4. Recovery from potassium starvation occurs the more slowly the longer potassium is withheld.
5. The relative distribution of potassium per gramme of dry matter formed as between tops and roots is the same whether the plants are growing in the presence of potassium, in the absence of potassium, or have first suffered from a more or less prolonged period of starvation.
6. When potassium-starved plants are supplied with potassium the element becomes distributed promptly in accordance with the physiological needs of the plant, the absorption progressing with marked rapidity.
7. The normal life cycle is inhibited in potassium-starved plants and more or less delayed by partial potassium starvation.
8. The relative potassium requirement per gramme of dry matter formed as between the tops and roots is the same in the case of wheat and corn, but in the case of buckwheat the requirement of the roots is higher than that of the tops.
9. The amount of potassium utilized per gramme of dry matter formed in normal plants of wheat and corn is substantially the same.
10. The amount of potassium utilized per gramme of dry matter formed in wheat and corn plants growing in the absence of potassium is substantially the same.
11. The amount of potassium utilized per gramme of dry matter formed in buckwheat growing in the absence of potassium is higher than in the case of wheat and corn.
12. The amount of potassium contained in the seed does not in itself determine the length of time a plant grown from it can live in the absence of potassium without injury resulting.

Transitional Herbaceous Dicotyledons.¹

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With Plates XI-XIII and five Figures in the Text.

A NUMBER of years ago, in one of the contributions issuing from this laboratory, Eames (1) described the origin of the herbaceous type in the Dicotyledons with special reference to the Rosaceae. The conclusions reached by this author vouched for the appearance of the herbaceous condition as the result of the formation of large storage rays in relation to the entering foliar traces. The evidence presented clearly indicates the derivation of these large foliar storage rays from the union of what were originally aggregations of more or less modified ordinary rays in proximity to the leaf-strands. In the midst of these aggregations of rays the vessels become transformed first to fibres and later, together with other originally elongated elements, into parenchyma. The parenchyma originating from the transverse septation of longitudinal prosenchyma becomes more and more assimilated with the radial parenchyma in its dimensions, and in extreme cases is scarcely distinguishable from it. It is clear, if the method of origin of foliar ray described by Eames for herbaceous representatives of the Rosaceae is correct, that herbaceous forms are derived from woody or arboreal ancestors. More recently, two authors working in collaboration (2) have expressed the opinion that 'Although this hypothesis accounts for many of the facts in the Rosaceae, it meets with difficulty when applied to other families and is open to criticism on several counts'.

The criticisms of the views put forward by Eames, which the authors describe as 'originating with Professor Jeffrey', are, in the words of their authors, as follows: 'In the first place, the transitional stages from a woody to an herbaceous condition, which it cites, and which form, indeed, the

¹ Contribution from the Laboratories of Plant Morphology of Harvard University.

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strongest evidence in its support, are found *not in the aerial parts of the stem but in the underground portions.*' Further, 'The fact that intermediate stages in harmony with the theory we are criticizing *are so rare in aerial stems as to be regarded as one of its weak points*'. In a following paragraph the authors under review state, 'The fact, however, which militates most strongly against the validity of the hypothesis under discussion is that, in practically all many-bundled herbaceous stems, the interfascicular parenchyma is not subtended by tiny leaf-trace bundles, nor is the stem composed of presumably typical alternating large and small bundles, the latter being leaf-traces.'

It will be shown in the sequel that Messrs. Bailey and Sinnott are apparently ignorant of fundamental facts in regard to the organization of the aerial stem in herbs. Further, they fail to distinguish the modifications in the general topography of the axis resulting from the different degrees of advance towards the herbaceous condition, thus showing a singular lack of capacity for thinking in three dimensions. The erroneousness of their statements will, moreover, be demonstrated on the identical forms which they present as evidence for the truth of their views. In advance, this general criticism must be made of their illustrations and their deductions. They fail to realize that if there is such a thing as a leaf-ray, it would naturally occur in the nodal region of the stem, where the traces enter the axial organ. Their illustrations throughout show a surprising inability to grasp this rudimentary principle of anatomy.

Obviously a clear conception of the differences of anatomical organization between a woody and an herbaceous stem can best be obtained by comparing the nodal regions of nearly related trees and herbs. The authors under discussion have figured *Hibiscus* and *Abutilon* as examples of herbaceous types. It may quite reasonably be urged that neither of these genera, which are of tropical or sub-tropical distribution, can be regarded as typical herbs, particularly by our authors; for in the later pages of their article they have elaborated at great length the hypothesis (which however did not originate with them) of the temperate distribution of characteristic herbs. Since both *Abutilon* and *Hibiscus* are distinctly woody and tropical herbs, they obviously will supply the supreme test of the correctness of this hypothesis, emphasizing the development of foliar rays as a fundamental feature of distinction between trees and herbs.

Fig. 1, Pl. XI, shows the general organization of a year-old stem of the common Basswood (*Tilia* sp.), or Linden, as an example of the nodal conditions in an arboreal representative of the Malvales. On either side of the long axis of the pith is to be seen a depression in the inner contour of the woody cylinder, marking the situation of a foliar trace, which has just entered the axis at the node above. In Fig. 2 a part of Fig. 1 is shown under a higher degree of magnification. The foliar trace lying in its internal

depression of the cylinder is obviously subtended externally by unmodified wood. The truth of this statement is confirmed by the inspection of Fig. 4, Pl. XI, which represents a tangential section through the foliar trace in its outward course through the wood. Above the trace lies a dark patch, the parenchyma of the leaf-gap, which is continuous internally with the medulla. The narrow pointed elements abutting on the lower border of the parenchymatous tissues of the leaf-gap are the tracheary elements of the foliar trace. Below the leaf-trace neither foliar ray nor any appreciable aggregation of the ordinary rays of the wood can be distinguished. It will be clear to the reader that nothing of the nature of a foliar ray is present in relation to the leaf-trace in the genus *Tilia*. Turning to the genus *Abutilon*, cited and figured by our authors in support of their view, we find in Fig. 5 a total transverse section of a year-old stem of *Abutilon* species. The plane of section is the same as in *Tilia*, namely, slightly below the node. The topography exhibited is, however, quite different, for here there are distinct parenchymatous interruptions in the continuity of the woody cylinder, three in number, corresponding to the three foliar traces entering the stem at the node. In Fig. 6, Pl. XI, one of these parenchymatous interruptions, or, as we prefer to call them, foliar rays, is shown under a higher degree of magnification. The organization of the structure in question obviously is entirely parenchymatous, and, contrary to the confident assertion of our critics, it is subtended internally by a small leaf-trace. Moreover, the node chosen for illustration belongs to the aerial stem, so that their contentions in every respect are proved erroneous on the basis of one of the genera chosen by them to illustrate the superior accuracy of their knowledge of the anatomy of Dicotyledonous herbs. Fig. 3, Pl. XI, illustrates the tangential aspect of the foliar ray in *Abutilon*. The leaf-trace is seen to be surrounded on all sides by copious parenchyma, constituting for the most part the substance of the foliar ray. A small portion of the parenchyma on the upper surface of the leaf-trace must, however, be conceded to the foliar gap. A very significant organization is presented by the lower region of the foliar ray. It will be observed that it passes gradually into the substance of the wood, and accompanying this transition is the progressive transformation of parenchymatous elements into fibres. This condition clearly indicates the fibrous origin of a considerable portion of the foliar ray. The rest of its substance must be regarded as composed of the fused normal rays of the wood. A very instructive comparison is that of Figs. 1 and 5, of Figs. 2 and 6, and of Figs. 3 and 4. In each case can be noted the relations of the leaf-trace to the organization of the cylinder in woody and herbaceous stems of close affinities. It probably will be quite clear to the reader that there is a distinct and interesting difference of organization between *Tilia* and *Abutilon*, which is most strikingly expressed in the development of specially large storage rays in the case of the latter, in relation to the entering traces

of the leaves. Our critics' figure of *Abutilon* is of indifferent quality, but is clear enough to show that it does not represent conditions in the nodal regions of the stem, and as a consequence has no bearing whatever on this discussion.

Turning our attention to the genus *Hibiscus*, illustrated by their Fig. 8, Pl. XXXIX, it is clear that foliar rays are present even in their indistinct and technically inadequate figure. In view of that fact it is surprising that they should deny the existence of foliar rays in the genus *Hibiscus*. In our Fig. 9, Pl. XI, is shown a total view of a three-year-old stem of the genus under discussion. On the right can be distinctly seen a foliar ray related to a corresponding trace and extending through three annual rings. Fig. 10, Pl. XI, shows a part of the leaf-ray more highly magnified. It is distinctly a structure differentiated from the normal organization of the wood by crowding of the ordinary rays and by the absence of vessels. The leaf-ray of *Hibiscus* is in fact a typical aggregate ray consisting of crowded wood rays separated from one another by fibrous bands, which include no vascular elements. The fibres contained in the aggregate leaf-ray of the genus under discussion represent a more primitive condition than that found in *Abutilon*, since the ray is here still in the aggregate condition throughout and has not, as in the case of the latter genus, been largely transformed into the homogeneous compound type of foliar ray, by the septation of the separating fibrous bands of the aggregation of rays into storage parenchyma. It seems clear that neither *Abutilon* nor *Hibiscus* justifies the sweeping statement of our critics as to the absence of foliar rays in the aerial stems of herbs of Malvaceous affinities. It is rather surprising that our authors did not have recourse to such common types as the Hollyhock (*Althaea*) and the various Mallows to support their assertions. An examination of these forms reveals a complete agreement with the data illustrated in our figures of *Abutilon* and *Hibiscus*. Among more exotic genera showing the same conditions as regards foliar rays may be mentioned *Sida*, *Napaea*, *Abelmoschus*, *Gossypium*, *Lavatera*, &c., &c. In concluding the statement regarding herbaceous representatives of the Malvales, we may appropriately reiterate that the aerial stems of these are distinguished from arboreal forms of similar systematic affinities by the development of distinct foliar rays in relation to the entering foliar traces. Other things being equal, these are the better developed the more advanced in the herbaceous scale are the plants under examination.

Before leaving the Malvales it will be well to devote some attention to the nature of the leaf-gap, since there is obviously some confusion in certain quarters on this subject. Fig. 7, Pl. XI, shows a part of the woody cylinder of a year-old stem of *Tilia* at the level where the foliar trace is beginning to enter the stem. Internal to the trace, which appears as a black area, is a radially directed mass of parenchyma ending in the pith. This is the

parenchyma of the leaf-gap. Such gaps are found more or less well developed in the secondary wood of all of the Pteropsida. On the outer (also the lower) side of the foliar trace there is no corresponding thick radial mass (see Fig. 2, Pl. XI) of storage parenchyma, except in forms provided with foliar rays (see Fig. 6, Pl. XI). Notable in this connexion, of course, are the Dicotyledonous herbs at present under discussion.

We may now consider with advantage another group in which herbaceous types occur side by side with arboreal. The Urticales will excellently serve in this connexion, because they are regarded by many, by reason of the chalazogamy which they manifest, as among the lowest of the Dicotyledons. Beginning as before with an arboreal representative, Fig. 13, Pl. XII, shows a complete view of a year-old stem of the American Elm, in the region of the node. The position of the traces is revealed by three marked depressions on the inner border of the pith. Fig. 14, Pl. XII, shows one of the leaf-segments of the woody cylinder, more highly magnified. It is clear that outside the leaf-trace the wood is practically normal in structure, showing the presence of wood rays, fibres, and vessels.

In Fig. 15, Pl. XII, is shown a total view of the nodal region of the thicker part of the stem of *Boehmeria nivea*, the so-called 'Fibre-Ramie'. This Urticaceous herb presents a marked contrast to the Elm in the nodal region, by reason of the presence of distinct and broad parenchymatous bands corresponding to the position of the leaf-traces in the stem. These are the foliar rays and are just as characteristically developed in the herbaceous representatives of the Urticales as they have been shown above to be in the Malvales. Fig. 16, Pl. XII, shows a detailed view of one of the leaf-segments of the stem and its corresponding foliar ray. The small leaf-trace is here again externally subtended by a well-developed parenchymatous leaf-ray in marked contrast to the generally woody organization of the central cylinder. The facts here, too, are as irreconcilable with the statements of our critics as they have been shown above to be for the Malvales. Fig. 17, Pl. XII, shows a higher region in the stem under a somewhat less magnification than that employed in the case of Fig. 16. In this illustration the foliar trace is still in the cortex and has not made its way into the central cylinder. A gap subtends the trace in the substance of the wood, which is of course the foliar gap. Fig. 18, Pl. XII, shows a tangential view of the leaf-ray in *Boehmeria*. It is quite obvious that the foliar trace is surrounded by a broad band of parenchymatous tissue, particularly well marked, below the trace. It is thus clear that the leaf-ray shows equally well in the tangential and in the transverse planes of section. In Fig. 19, Pl. XII, is shown a much more magnified view of the foliar ray in the species under discussion. With the increased magnification it becomes clear that the leaf-ray still reveals vestiges of its ligneous origin by the presence of fibres more or less distinctly developed. Examples from herbaceous representatives of the

Urticales of the presence of foliar rays might be multiplied, but the single illustration will suffice, particularly as one of us has recently figured the similar condition in the common Stinging-Nettle, *Urtica urens* (3).

Before taking leave of the Urticales, it will be well to figure for the *Fibre-Ramie* the result on the organization of the leaf-ray of the thinning of the stem in its upper region. Fig. 11, Pl. XI, represents an entire nodal transverse section of *Boehmeria nivea*. The leaf traces are seen, three in number, as in the stouter region of the axis. Their topographical relations are, however, modified as a result of the thinner woody cylinder. Since the cylinder of wood does not appreciably exceed in radial dimension that of the foliar trace, the mass of parenchyma radially subtending the foliar trace in the lower and thicker region of the stem is necessarily absent and the foliar storage tissues are represented only by the parenchyma, which flanks the leaf-trace on either side. This flanking parenchyma compensates for its reduced radial development by a correspondingly increased longitudinal extension. As a consequence of this condition, the cylinder in Fig. 11, Pl. XI, is much more clearly broken up into distinct bundles (naturally separated from one another by foliar rays) than is the case with the more woody lower region of the axis shown in Fig. 15, Pl. XII. Fig. 12, Pl. XI, shows a portion of Fig. 11, much more highly magnified. The foliar trace lies in the centre, and occupies an outstanding position in regard to the strands of the stem on either side, from which it is separated by the flanking foliar parenchyma.

Fig. 20, Pl. XII, shows a complete view of the nodal region of a four-year-old axis of the genus *Xanthorhiza*. This Ranunculaceae genus has been figured by the authors (*op. cit.*, Fig. 12) of the article referred to above as furnishing evidence in favour of their view that there are no foliar rays in the aerial axis of herbaceous forms. It is clear from our illustration of the stem of this somewhat woody herbaceous representative of the Ranunculaceae that there are numerous radial bands extending through all the four annual rings and corresponding to dark radially directed structures, the leaf-traces. Fig. 21, Pl. XII, shows a detail of a three-year-old stem of the same species including three foliar traces and their corresponding leaf-rays. To make the situation still more clear, a tangential view of two foliar rays with their included foliar traces is shown in Fig. 22, Pl. XII. It is thus abundantly clear that the genus *Xanthorhiza*, selected by our critics to illustrate the absence of foliar rays in the Ranunculaceae, serves them very badly in this respect, since well-developed rays are seen not only in sections through the nodal region, but almost equally well in those which pass through the internode. In fact, it may be stated that, except in the genus *Paeonia*, which is to be regarded as perhaps the most primitive representative of the Ranunculaceae proper in the Northern Hemisphere, the only rays present in the woody cylinder are those to which we have applied the

name foliar. In *Paconia* there are smaller rays of the normal woody type, as well as those of broader dimensions, belonging to the foliar category. It will be obvious to the reader that the authors under discussion have not been very fortunate in their choice of the Ranunculaceae as a group demonstrating the accuracy of their statements. It may be added that any of the Ranunculaceae possessing a reasonably well developed aerial stem clearly show the presence of foliar rays. This, for example, is true of both *Clematis* and *Thalictrum*. In the more advanced herbaceous species the situation is less obvious by reason of the thinning of the woody cylinder. In many cases, however, the situation is revealed by the anatomy of the perennial subterranean stem. It is true that our critics have rejected the evidence furnished by the anatomy of the terrestrial region of the stem in extremely herbaceous perennials. This attitude, however, rests on a fundamental misconception, which need not be enlarged upon at the present time. In the arboreal representatives of the Ranales the leaf-ray is in general as conspicuous by its absence as it is in the tree-like examples of other natural families, including both woody types and herbs.

At this stage it will be convenient to refer to a statement quoted at the beginning of this article. It is as follows: 'The fact, however, which militates most strongly against the validity of the hypothesis under discussion is that, in practically all many-bundled herbaceous stems, the interfascicular parenchyma is not subtended by tiny leaf-trace bundles, nor is the stem composed of presumably typical alternating large and small bundles, the latter being leaf-traces.' Fig. 23, Pl. XII, represents a transverse section of *Euphorbia Cyparissias*, the common garden Spurge. The margin of the medulla is crenulated by projections of the pith into the woody cylinder. Each of these bays harbours a leaf-trace which shows as a small dark dot terminating the apex of the bay. It is clear that outside each of the leaf-traces lies a well-developed foliar ray, and that these foliar rays clearly alternate with the stem segments, which are quite obviously characterized by the presence of vessels, conspicuous by their absence in the rays. It will be clear to the reader that the illustration under consideration very obviously realizes a condition stated by our self-confident critics not to exist. Almost any species of Sunflower, Aster, or Golden-rod, if sectioned in the appropriate region of the stem, would reveal similar topographical relations. In fact, hundreds of examples could be easily supplied of conditions which are cited above as impossible. The situation emphasized reveals the superficiality which unfortunately characterizes much of the recent anatomical work of the senior of our two critics.

Fig. 24, Pl. XII, exemplifies the anatomical conditions obtaining in the region of the foliar trace in *Robinia Pseudacacia*. The leaf-trace is opposed by normal wood, and there is consequently no foliar ray present in this arboreal representative of the important Legume alliance. In contrast to

Fig. 24, we find in Fig. 25, Pl. XII, a leaf-trace subtended by a remarkably well developed foliar ray. The species used here is the herbaceous *Melilotus alba*. The occurrence of foliar rays is practically universal in herbaceous and vine-like representatives of the Leguminosae, and they consequently supply good evidence in favour of the coincidence of foliar rays with the herbaceous type. Here, as elsewhere, when the aerial stem is extremely slender, a radially well developed foliar ray is found only in the thick perennial subterranean axis.

In a group like the Umbelliferae, the representatives of which in temperate regions are almost exclusively tender herbs, it is usually possible to discover radially developed foliar rays only in the persistent hypogaeous axis. The same statement holds true for a large number of other highly herbaceous orders. Obviously, in seeking a derivation of the herbaceous type from the woody, it is necessary to choose types of herbs in the first instance which are more or less woody in order to successfully trace the stages from the arboreal to the herbaceous type. Our critics have made the mistake of comparing extreme herbs with trees, and have failed to investigate with even moderate care the more important transitional forms which unite trees and herbs.

Fig. 26, Pl. XIII, illustrates the organization of the nodal region of the Scrophulariaceous genus *Gerardia*. The leaves are opposite, and corresponding to these are two opposite foliar traces. The trace on the lower side of the figure is making its way in towards the centre and is consequently subtended inwardly by the foliar gap. The upper trace by contrast has reached its final position on the margin of the pith and subtends radially a well-marked parenchymatous band, the foliar ray. In Fig. 27, Pl. XIII, is shown a detail of the foliar ray, and its contrasted structure may be compared with the ordinary wood on either hand. Almost any of the Scrophulariaceae show similar conditions as to the development of foliar rays, notably the common Mullein (*Verbascum*) and the Toad-flax (*Linaria*).

In conclusion, we may deal with the Compositae. Fig. 28, Pl. XIII, illustrates a transverse section of the foliar ray in *Solidago canadensis* from a preparation made by Miss Edith Whitaker, who has recently published an interesting contribution on the anatomy of the Golden-rods (4). A well-developed radial mass of storage parenchyma is revealed in the section. Fig. 29, Pl. XIII, also from a preparation by Miss Whitaker, reveals the tangential aspect of the foliar ray. The trace in this instance, as is indeed common in the Compositae, lies well to the top of the ray. In Fig. 30, Pl. XIII, is shown the radial view of the foliar ray. This illustration is particularly instructive. Above is shown the ordinary structure of the wood which is normally found above the region of exit of the trace in stems which are not too extremely herbaceous. Below the foliar trace, which shows as a structure pursuing an oblique and rising curved course towards

the left, lies the radial aspect of the entirely parenchymatous foliar ray. To the right of the leaf-trace is shown the darker-hued parenchymatous material, which represents the soft matrix occupying the leaf-gap. The three figures described above give an accurate view in three dimensions of the foliar ray as it appears in the Golden-rod. The topography of the foliar rays is surprisingly uniform in the Compositae, and literally hundreds of instances could be furnished for illustration, exactly paralleling those shown in Figs. 28, 29, and 30. Figs. 31 and 32, Pl. XIII, show tangential views of the foliar rays in two different species of Sunflower. In Fig. 31 is revealed the tangential aspect of the leaf-ray in *Helianthus tuberosus*, a perennial species with annual aerial stems. In the substance of the leaf-ray in this species even the low power of magnification used reveals the presence of other than parenchymatous elements. In other words, the ray is of mixed constitution and still contains some of the vessels and fibres of the wood from which it was originally formed. In Fig. 32, Pl. XIII, is revealed, under the same degree of magnification as in the preceding figure, the foliar ray of the strictly annual species *Helianthus annuus*. The very much larger size of both foliar ray and its corresponding foliar trace are easily observed. The difference in dimensions observed corresponds closely with the degree of herbaceousness in the two species. *Helianthus annuus* is large, vigorous, and very herbaceous, and is able to go from seed to seed in a comparatively few weeks. Another feature of interest from the comparative standpoint, other than the greater development of both leaf-trace and the corresponding foliar ray in *H. annuus*, is the homogeneity of the ray itself, indicating a more advanced degree of transformation from the original wood into storage tissue.

In the foregoing paragraphs and their accompanying photographic illustrations a considerable variety of evidence has been supplied in support of the proposition advanced from these laboratories some years ago, that herbaceous stems, in their origin at any rate, are characterized by the presence of large foliar rays, radially subtending the foliar traces. It should be emphasized that the figures are only illustrative of an infinitely larger number of facts of a similar character, as it is obviously impossible within the limits imposed by an article in a scientific magazine to describe or cite in detail more than a comparatively few cases bearing upon the theme under discussion. An attempt has, however, been made to have the illustrations chosen thoroughly representative of the course of evolution of herbs from woody ancestors. In a good number of instances the woody or arboreal type has been figured in proximity to its herbaceous derivative so that easy comparison of the two is possible. The present authors are of the opinion that, with these illustrations before his eyes, no competent and fair-minded reader can deny the existence in typical Dicotyledonous herbs of the structures which we have called foliar rays. It will further be clear to those

who peruse these lines, it is hoped, that the foliar ray, practically universally characteristic of Dicotyledonous herbs, is a compound structure derived from the aggregation of storage tissues in the region of the foliar trace, as it enters the stem at the node.

Before passing on to the diagrammatic illustrations of the derivations of transitional herbs from woody ancestors, it will be well to pass in review the figures of the article (1) which it has been necessary to discuss in the present pages.

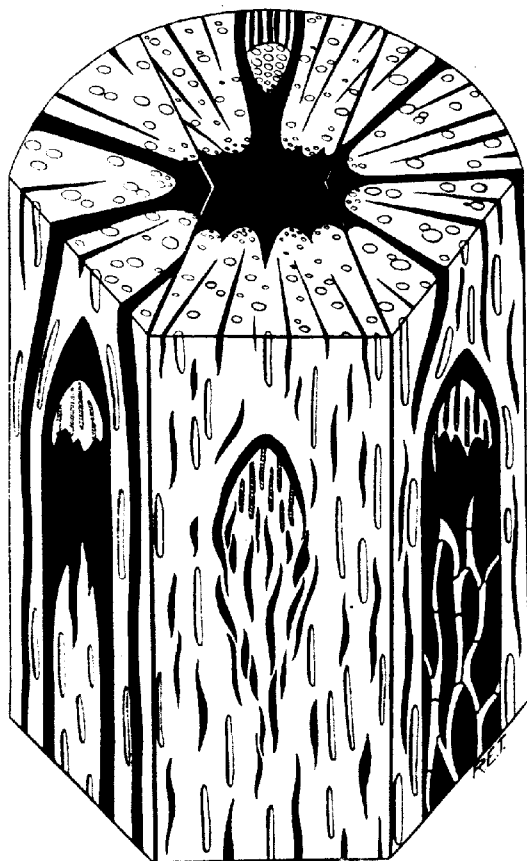
Their first illustration of the Compositaceous genus *Arctotis* is an excellent example of the peculiarly inconsistent reasoning which characterizes the anatomical statements of our critics. After objecting strongly to the use of the decumbent stem of *Potentilla palustris* by Eames (1) in his article proceeding from these laboratories, they use the "somewhat decumbent aerial stem of *Arctotis grandis*" in support of their own conception of the origin of the herbaceous type. Fig. 2 of their article represents the stem of the American Beech and has no bearing on the present discussion. Fig. 3, of *Hypericum aureum*, is not taken in the region where the leaf-traces enter the stem at the node, and consequently has no bearing on the matter in dispute, namely, the presence of foliar rays in relation to foliar traces. We have examined several species of *Hypericum* and have invariably found foliar rays related at the node to leaf-traces. The following figure of another species of *Hypericum* is open to the same criticism as Fig. 3. The next figures (5 and 6) illustrate the stem of the Solanaceae, but not at the node. Here, as in the Hypericaceae, well-marked foliar rays are to be seen where the leaf-traces enter the stem. Fig. 7, of *Sida* sp., may be taken as illustrative of the Labiatae, which are as well characterized by the presence of foliar rays as are other herbaceous orders. Figs. 8 and 9, illustrating the Malvales, have been sufficiently criticized in the foregoing pages. Fig. 10, of the stem of *Rosa rugosa*, has little bearing on the discussion. The authors do not figure any herbaceous representatives of the Rosaceae, with the exception of *Sanguisorba*, in which foliar rays are clearly present even in the aerial stem, although not shown in their illustration. The futility of their statements regarding the Ranunculaceous genus *Xanthorhiza* has been revealed in earlier paragraphs. The other illustration of the Ranunculaceae, *Delphinium*, is through too slender a stem to throw any light on the question of the origin of herbs from woody ancestors. *Acanthopanax* shows some progress towards the herbaceous condition and the formation of foliar rays, although this is not manifest in the illustration supplied by our critics. Fig. 15, illustrating the Umbelliferae, is made through the internode of an extremely herbaceous axis, and as a consequence throws no light whatever on the origin of the herbaceous type from the woody, which is naturally best elucidated by herbs of intermediate and transitional organization. Figs. 16 and 17 are characterized

by the same faults as many of the others, namely, they are not taken properly in the nodal region or the stems are too slender to reveal the condition of transition of fundamental importance in the present connexion. These shortcomings are all the more serious because the two illustrations in question are all that the authors have presented for the very important herbaceous group, the *Gompositae*. Fig. 18, a further example of the *Ranunculaceae*, is not from a nodal region of *Clematis*, and as a consequence naturally throws no light whatever on the typical foliar ray, which in woody herbs like the species of *Clematis* illustrated is of shallow vertical length and confined as a consequence to the region immediately below the node and the trace to which it is related.

The elucidation of the results reached by the examination of transverse and longitudinal sections of transitional herbs is naturally best accomplished by means of diagrams. Text-fig. 1 shows at once the tangential and transverse aspects of three different genera of the *Compositae*. In the centre appears a representation of the stem of the southern woody genus *Baccharis*, in the region of the node. The leaf-trace is diagrammatically indicated by elements with spiral markings. It will be noted in the tangential aspect which faces the observer that there is only a slight concentration and enlargement of the rays of the wood in proximity to the foliar trace. On the opposite side of the transverse aspect of the central region of the diagram is shown the transverse section of a leaf-segment of a higher node. Obviously we have here to do with an aggregate ray related to the leaf-trace, since no vessels are present in the region of the cylinder radially subtending the vascular supply to the leaf. To the right in the diagram appears the nodal tangential aspect of *Bidens* sp. Here there is a considerable fusion and enlargement of the normal rays of the wood in relation to the foliar trace, and as a consequence the storage conditions are farther advanced than they are in the central segment of the diagram. No transverse section of the foliar ray is represented in the case of *Bidens*. To the left in the diagram is shown the tangential aspect of the nodal situation in the genus *Helianthus*. The storage devices here have become better developed than in *Bidens*, since a well-marked compound and as a consequence purely parenchymatous ray has made its appearance in relation to the foliar trace. A notable feature in this instance is the presence of a central tongue of unmodified wood in the lower region of the foliar ray. This structure has the effect of subdividing the leaf-ray into two in the lower part of its course. The two narrow forks of the foliar ray in many herbs run for a long distance down the stem, and as a consequence through a number of internodes. The vertical length of the foliar ray other things being equal, has a direct relation to the degree of herbaceousness of the axis. It may vary in the same stem, for in the lower aerial region, where the cylinder is thick and woody, the vertical extension of the

foliar rays may be short, while the radial development is often prominent. In the upper slender portion of the same axis, on the other hand, the rays are frequently shallow radially and vertically extremely elongated.

In Text-fig. 2 are elucidated, in respectively more and less herbaceous species of *Clematis*, the topographical relations of the foliar rays. The

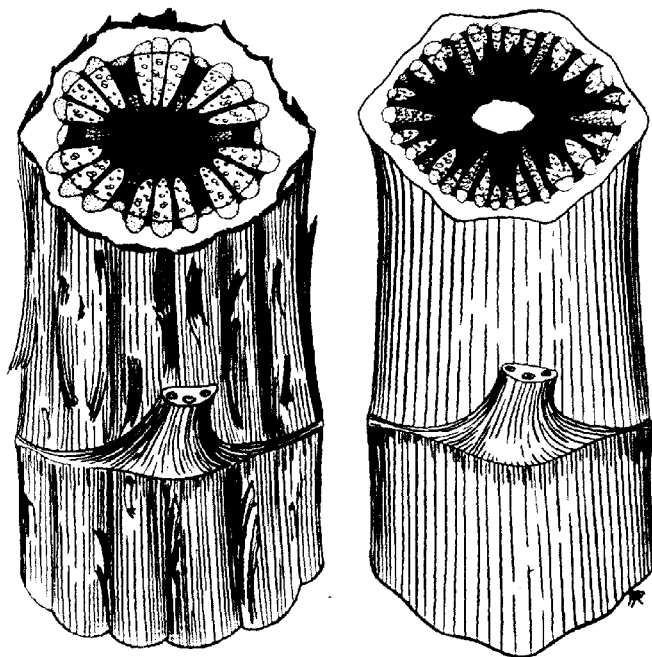


TEXT-FIG. 1. Centre diagram of the woody genus *Eaccharis*. Left, tangential view of the node of *Hibenthia*. Right, tangential view of the nodal region of *Bidon*.

diagram to the left shows a somewhat woody species. There are six foliar traces projecting somewhat into the medullary region, to which are related radially six corresponding foliar rays. The storage tissue in these rays is mainly exterior to the foliar traces, but some of it lies on their flanks. In the right-hand figure an herbaceous species of *Clematis* is represented. Here the

six foliar traces are relatively very much larger in size than they are in the companion illustration, a frequent condition of contrast between woody and herbaceous axes in the same genus. The six leaf-traces of the herbaceous stem are of such marked radial development that the foliar storage tissue is absent radially, and as a consequence is confined to a lateral position on the flanks of the traces of the leaves in their course in the stem.

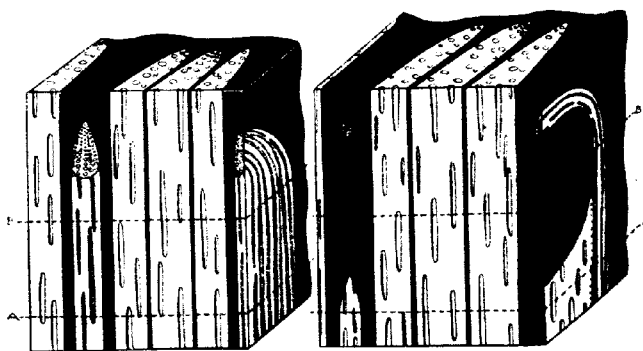
Text-fig. 3 shows the situation as regards the relation of the storage rays to the foliar traces in three dimensions. The left-hand diagram repro-



TEXT-FIG. 2. Diagram of the nodal regions of a woody and an herbaceous species of *Ulm*.

duces the topographical relations of leaf-trace and foliar ray in the slender herbaceous region of the stem. To the left of the illustration lies a foliar ray with its contained leaf-trace. The trace is represented below in tangential long section, while above, on account of the fact that it turns obliquely outwards or radially in its course towards the base of the leaf, it is seen in somewhat oblique transverse section. In the transverse view of the ray is shown a solid mass of storage tissue representing the enlarged foliar gap. To the right of the diagram under discussion is shown the radial

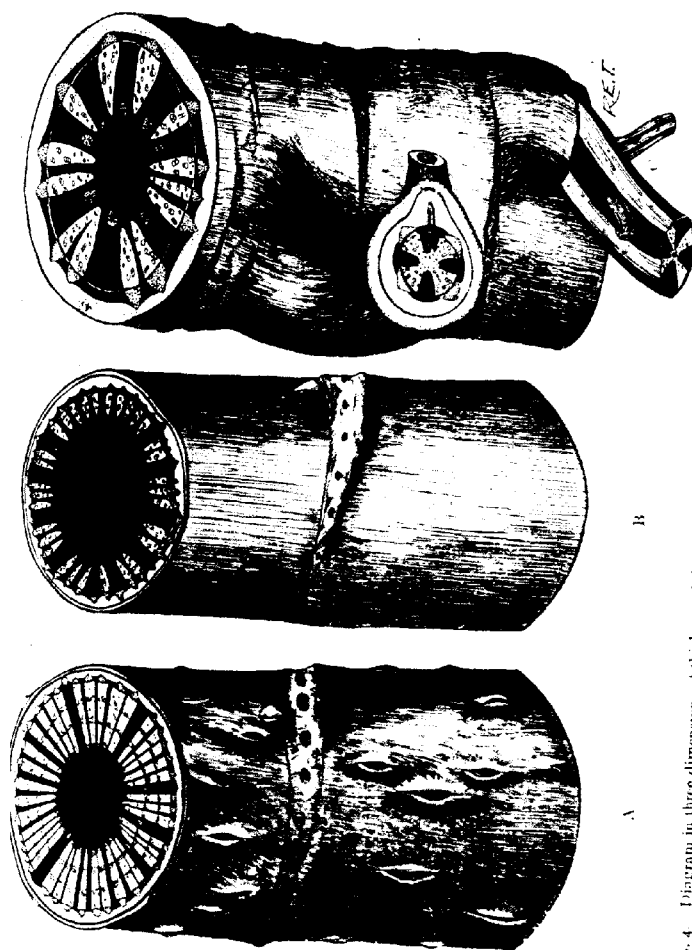
aspect of the woody cylinder in a segment occupied by a foliar trace. The relations of the ray to the trace can be clearly seen and also the fact that the foliar trace turns somewhat abruptly radially outwards at its upper extremity. In the diagram to the right the topographical relations of the foliar trace in a more woody herbaceous axis are shown, in the region of the node. To the left the foliar trace is seen only in transverse section in the tangential plane, since its vertical portion is buried deeply under the parenchyma of the foliar ray. In the lower region the massive foliar ray assumes the divided condition, which is present from the first in thin axes, below the node. The transverse view of the stem shows the large foliar gap so characteristic of the herbaceous type, whether of soft or woody texture. The right side of the figure under discussion shows the radial aspect of the foliar trace and of its related foliar ray. The trace in



TEXT-FIG. 3. Diagram of the topographical relations of leaf-trace and foliar ray in three dimensions. Left, a more delicate herbaceous stem. Right, a stem of more woody development.

its vertical course is buried below under the central tongue of xylem, bifurcating the foliar ray in its lower portion. Higher up the leaf-trace comes to lie under the tangential portion of the foliar ray, which is not present in the more slender cylinder represented in the left-hand item of the diagram. In its uppermost course the leaf-trace passes rapidly outwards, and in this region is completely surrounded by storage tissue.

Text-fig. 4 presents in its three items the general topography of thick and thin axes of the same somewhat woody aerial stem and of the more parenchymatous subterranean stem of a herbaceous perennial. A and B are constructed from thick and thin aerial stems of *Xanthorhiza*. In C appears the rhizome of a species of *Actaea*. The illustration in the last instance shows also the topography of the attached roots and their corresponding rootlets. Beginning with A, we have a stem three years old, shown in the transverse section in the region of an upper node. Below is the superficial view of another node. From this part of the diagram it is



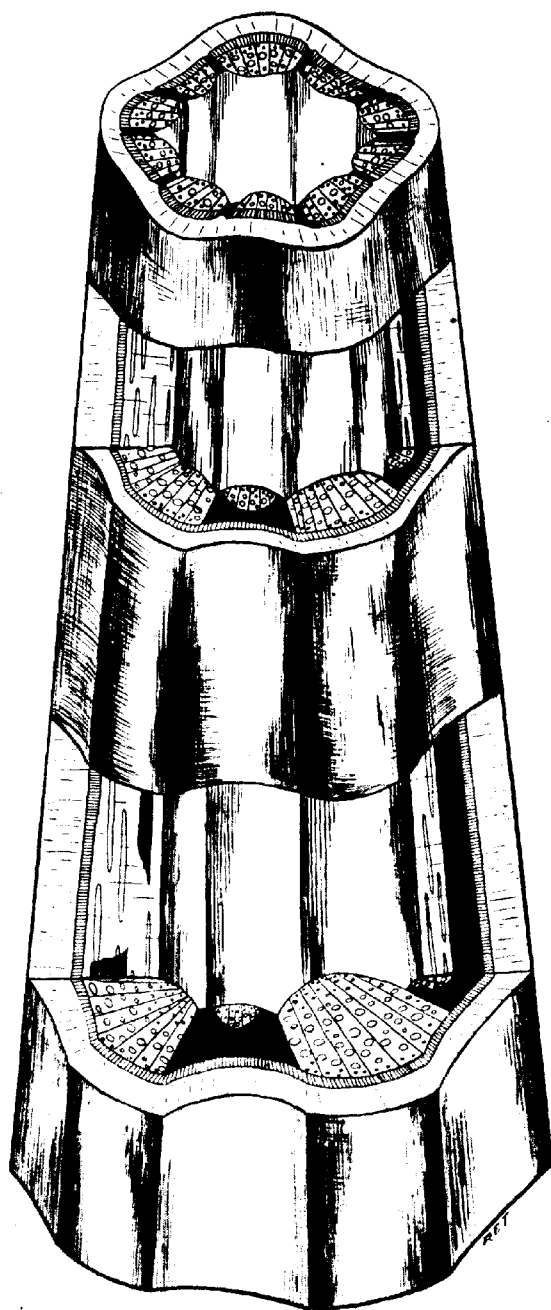
TEXT FIG. 4 Diagram in three dimensions of thicker and thinner aerial stems of *Xanthoxila*. A and B. Diagram of the root-stock of a species of *Alata*, showing the topography of the rays in relation to appendages of both root and stem (C).

clear that many foliar traces enter the stem at the same node, but at somewhat different levels. The upper transverse view shows the traces in topographical relation to the foliar rays. It is clear that the latter extend through three annual rings and subtend the traces as long radial and massive bands of storage tissue. Foliar rays are thus distinctly present in the aerial axis of *Xantherhiza*, contrary to the erroneous statement cited in an earlier page. They may extend through four or even five annual rings, and are such a striking feature of the topography of the stem that it is difficult to conceive how they could escape notice on the part of even an anatomical tyro. Diagram B shows the same general relations in a slender annual axis of *Xantherhiza*. Here the foliar traces are practically of the same radial extent as the bundles of the cylinder, and as a consequence the tangential part of the foliar rays is absent. The result of this situation is that the only portions of the leaf-ray to be present are those flanking the foliar trace itself. It is necessary, however, in order to understand the conditions present in B, to have in mind those exemplified in A. The herbaceous stem in the latter has well-marked foliar rays developed both in a flanking and a radially confronting position. In progressively more slender stems, as a necessary geometrical result of the thinning of the axis, the confronting portion of the ray is progressively reduced and finally disappears altogether. Obviously, if the slender herbaceous type is derived from the more woody herbaceous axis, and this in turn from the normal arboreal type, the topographical relations of the storage tissues related to the foliar traces should be studied in the order of arboreal, woody herbaceous, and slender herbaceous. No other procedure is permissible if herbs are in reality derived from woody ancestors in the Dicotyledons.

But it is in Diagram C of Text-fig. 4 that the greatest difficulties to the views recently put forward in this Journal (2) are to be seen. In the axial portion of the illustration a number of foliar traces can be seen in proximity to the medulla. These do not all belong to the same leaf, since the foliar organs are somewhat crowded on the subterranean stem. Each foliar trace is related to a well-marked foliar ray, which manifests both flanking and confronting relations to the trace. It is to the roots, however, that we particularly turn our attention in this diagram. In the upper root appearing in the illustration the organ is shown both superficially and in transverse section. In the centre of the latter aspect appears a four-angled protoxylem star, subtending the projections of which are four well-marked parenchymatous interruptions of the secondary wood. These are the root rays, which are often just as characteristically developed in relation to the lateral rootlets as are similar storage rays in the stem in relation to the foliar traces. Since the woody cylinders of the lateral rootlets are from the first horizontally directed from their point of departure on one of the angles of the protoxylem, they are clearly surrounded on all sides by

storage tissues, and the geometrical complexities which are indicated in the case of the stem do not present themselves. Moreover, since the root is a somewhat conservative organ, it, in many cases at least, indicates the primitive conditions of the herbaceous type of organization, characterized normally in both stem and root by large rays related to the appendages. The lower root in the figure is shown in tangential section, so that the very definite relation of the secondary root to a large storage ray in the main root can readily be made out. The conditions presented in this last item of our diagram could easily be duplicated for *Clematis*, *Thalictrum*, and many other herbaceous representatives of the Ranales. Moreover, this is a situation of very wide occurrence in herbaceous forms of the most diverse systematic affinities. It is accordingly clear that, so far as the organization of typical herbaceous roots is concerned, the evidence is as conclusively against our critics as it is in the case of the axis.

Text-fig. 5 is intended to show the topographical relations of leaf-trace and leaf-ray in an aerial axis of the genus *Potentilla*. The statement has been made, in the article to which we have been under the necessity of so often referring in the present pages, that only in the subterranean stem of the herbaceous representatives of the Rosaceae do the structures which we designate foliar rays occur. This assertion is unfortunately as devoid of foundation as are many of the statements of fact of our critics. *Sanguisorba*, *Agrimonia*, and *Potentilla*, as well as a number of other herbaceous Rosaceae, manifest clearly developed foliar rays in their aerial axes. The diagram shows the modifications in organization of the foliar ray in passing from the lower to the upper regions of the aerial stem. The cylinder is represented as cut away in the regions below three successive nodes. In the lowermost of these the foliar trace is shown on the margin of the medullary region. Here the leaf-trace is much smaller in its radial dimension than is the cylinder to which it belongs. It is related to a large amount of storage tissue, most abundantly developed outside the trace, but also flanking it on either side. The next level indicates a somewhat narrowed cylinder in which the foliar trace as a consequence bulks more largely in radial relation. As a necessary geometrical consequence of this situation the confronting portion of the foliar ray is considerably reduced. At the top of the figure appears the most slender aspect of the axis. Here the woody cylinder has on the one hand become reduced in thickness, and on the other the foliar trace has increased somewhat in radial development. These concurrent conditions lead automatically to the complete elimination of that portion of the foliar ray which lies radially external to the foliar trace. The flanking portions of the foliar ray, however, are still present, and represent the foliar storage provision for this region of the axis.



TENT-FIG. 5. Diagram to illustrate the topographical relations of the leaf-trace to the leaf-ray at various heights in the tapering aerial stem of *Potentilla*.

CONCLUSIONS.

The various photographic illustrations and stereodiagrams supplied in connexion with the earlier pages of the present article seem clearly to justify quite definite conclusions as to the mode of origin of the herbaceous type of axis in the Dicotyledons from that of woody or arboreal texture. It seems obvious, as a consequence of the comparison of nearly related arboreal, woody herbaceous, and slender herbaceous stems, that the structures which we have called foliar rays are in the first place developed in woody herbs as a result of the clustering of ordinary rays of the wood in relation to the incoming leaf-traces. The clusters or congeries of rays, to which one of us has applied the name aggregate rays, are often characterized by the fact that vessels are eliminated in the bands of longitudinal woody elements separating the constituent members of the foliar aggregation of rays from one another. We have illustrated this condition above, in the case of the genus *Hibiscus*. It is of wide occurrence in woody or transitional herbs. A next step in the development of the herbaceous type is the transformation of the strands of fibres separating the storage units of the aggregate ray from one another into rows of parenchymatous elements which become more and more assimilated to the ordinary radial parenchyma both in their dimensions and in the relations of their axes. This condition of the foliar ray we have called the compound ray, to distinguish it from the aggregate ray from which it takes its origin. Quite often when the compound ray has been thoroughly established it still betrays its derivation from the aggregate ray by the fact that its lower portion is still largely in a condition of aggregation. As the herbaceous condition becomes more and more established, the foliar rays become not only more homogeneous, by reason of the more complete loss of identity of their originally diverse woody elements, but also more elongated in the vertical direction.

The last condition often expresses itself in transverse section, where the traces are sufficiently numerous and their accompanying foliar rays are sufficiently developed in the long axis, by a series of separate strands, more or less regularly alternating as to size, in which certain members are deep radially and woody in structure, while the alternating segments consist of relatively slender bundles subtended radially by massive storage tissues. The storage tissues also occur on the flanks of the slender bundles just referred to, which are the foliar traces in their course in the stem. This state of affairs is extremely common, for example, in the more woody lower region of the aerial stem in the herbaceous Compositae, particularly if the foliar traces are numerous and the foliar rays greatly extended longitudinally. Favourable objects on which to test the truth of this general statement are the genera *Helianthus*, *Aster*, *Lactuca*, &c., &c. Although the organiza-

tion just referred to is particularly well seen in Compositaceous stems of the proper degree of advance in the herbaceous direction, it also occurs in many other groups, where the necessary conditions are realized. It is a striking example of the inaccuracy which we have often had to note in our critics that they deny the possibility of the topography which we have represented photographically in Figs. 20 to 23 for the Ranunculaceae and the Euphorbiaceae. Hundreds of similar cases could be supplied from common herbaceous types.

Another and more advanced phase of the evolution of the herbaceous type is the thinning down, in an even more marked degree, of the axis. This automatically results in the elimination of the parenchyma of the foliar ray, which radially subtends the foliar trace. As a consequence of this condition, only the flanking parenchyma of the foliar ray persists. This situation is shown in our Figs. 11 and 12, and also by Text-figs. 3, 4, and 5.

It has been maintained by our critics that the herbaceous type in the Dicotyledons is merely the result of the progressive thinning of the woody cylinder. This condition is undoubtedly true for some Vascular Cryptogams of herbaceous habit, but cannot be accepted for the Seed-plants. The Coniferales, for example, have never given rise to plants either herbaceous or vine-like, in spite of the fact that they exist often under climatic conditions extremely favourable as regards low temperature to the appearance of the herbaceous habit. The reasonable inference from this state of affairs is that the Conifers lack some fundamental feature of organization which is essential to the development of the herbaceous type among the Seed-plants. We are by no means compelled, however, to take refuge in negative evidence in this respect, for it has been shown by numerous examples drawn from woody or transitional herbs, in the foregoing pages, that a constant and in fact diagnostic feature of the herbaceous type in the Dicotyledons is the presence of those structures which we have designated foliar rays. As explained above, these arise in the first place and in many woody herbs from the accentuation of the ordinary rays of the wood in proximity to, and especially below, the entering foliar traces. These aggregations of rays, by the process of compounding, are transformed later into considerable foliar bands of longiradial storage tissue. It follows from the statement of the manner of origin of herbs in the Dicotyledons that it is the result of advance and differentiation, and is not the consequence of a mere process of degeneracy, as assumed by our critics. Angiosperms are, in fact, as has been pointed out by one of us in a recently published text-book of anatomy, characterized by the development of *dynamic* herbs in contrast to the Vascular Cryptogams, which are represented in the living *Flora* by *degenerate* herbs.

SUMMARY.

1. The origin of the herbaceous type in the Dicotyledons is from woody or arboreal forms.
2. Woody herbs, as a consequence, throw clear light on the mode in which the herbaceous Dicotyledons have been derived.
3. In the aerial axes of woody herbs a constant and practically never-failing distinction from trees is the formation of large foliar storage rays about the incoming leaf-traces, as they pass through the woody cylinder.
4. In woody herbs the foliar storage rays are well developed in the radial direction, but their vertical extension is slight.
5. In the aerial stems of more slender and less woody Dicotyledonous herbs the foliar rays become elongated vertically to compensate for their reduced radial dimension resulting from the thinning down of the woody cylinder.
6. In rays of the type described in 5, the lower part of the radial parenchyma related to the foliar trace is often bifurcated by a tongue of unmodified wood.
7. The vertical elongation of the foliar rays and their subdivision in the manner described in 6 result in the final separation of the originally continuous woody cylinder into a series of separate strands.
8. The final stage of the herbaceous Dicotyledons is a condition in which the cylinder is thinned to such a degree that the radial extension of the foliar rays is virtually eliminated. With this condition is usually associated a great development in length of the portions of the foliar ray flanking the leaf-trace on either side.
9. Recent statements asserting the absence of foliar rays in the aerial axis of the herbaceous type in the Dicotyledons are inaccurate.

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EXPLANATION OF PLATES XI-XIII.

Illustrating Messrs. Jeffrey and Torrey's paper on Transitional Herbaceous Dicotyledons.

PLATE XI.

Fig. 1. Transverse section of a year-old stem of the common Linden (*Tilia*) in the region of a node, showing the absence of foliar rays such as are characteristic of woody herbs.

Fig. 2. Foliar segment of Fig. 1, more highly magnified to show the woody character of the tissues subtending the foliar trace.

Fig. 3. Tangential section of the foliar ray of the woody herb *Abutilon*.

Fig. 4. Tangential section of the foliar segment in *Tilia*, showing the absence of the foliar ray in this genus.

Fig. 5. Entire transverse section of the stem of *Abutilon* sp., showing clearly the presence of three foliar rays, corresponding to the three foliar traces on the margin of the pith.

Fig. 6. One of the foliar rays of *Abutilon* in transverse section, more highly magnified than Fig. 5. Compare this with the transverse section of *Tilia* in Fig. 2.

Fig. 7. Transverse section through part of the nodal region of the woody cylinder of *Tilia* sp., showing the foliar gap which interrupts the continuity of the wood internally to the leaf-trace.

Fig. 8. Transverse section of the foliar ray of *Oenothera biennis*, taken from the aerial region of the stem.

Fig. 9. Transverse section of the entire axis of *Hibiscus syriacus* in the region of the node. On the right is seen a foliar ray extending through three annual rings.

Fig. 10. Transverse section of part of the foliar ray shown in the preceding figure. The ray is seen to be made up of normal wood rays and of fibres, but vessels are conspicuous by their absence.

Fig. 11. Total transverse section of the slender region of the aerial axis of *Boehmeria nivea* in the region of the node.

Fig. 12. Part of the same more highly magnified to show the nature of the foliar rays in the slender region of the stem.

PLATE XII.

Fig. 13. Transverse section of the nodal region of a year-old stem of the American Elm (*Ulmus americana*) to show the absence of foliar rays in an Urticaceous woody axis.

Fig. 14. A foliar segment of *Ulmus americana*, and its internally subtending foliar trace. The absence of modification in the woody structure of the leaf-segment is very obvious.

Fig. 15. Entire transverse section of the nodal region of the stem of *Boehmeria nivea*, a woody herb of Urticaceous affinities, showing, in contrast to Fig. 13, the obvious presence of foliar rays in relation to the foliar traces.

Fig. 16. One of the foliar rays of *Boehmeria nivea* in transverse section. It is subtended internally by its corresponding foliar trace.

Fig. 17. Transverse section of the foliar gap of *Boehmeria nivea*. The leaf-trace subtends it externally in the cortex.

Fig. 18. Tangential section of the foliar ray of *Boehmeria nivea*.

Fig. 19. Part of the same, more highly magnified.

Fig. 20. Total transverse section of the aerial perennial stem of *Xanthorrhiza*, showing numerous foliar rays and their corresponding foliar traces.

Fig. 21. Part of a transverse section of the same species, more highly magnified to show three foliar rays extending through three annual rings and subtended by their corresponding foliar traces.

Fig. 22. Tangential section of two foliar rays of *Xanthorrhiza*.

Fig. 23. Transverse section of *Euphorbia cyparissias*, showing a clear alternation of numerous stem bundles and of foliar rays subtended by their foliar traces.

Fig. 24. Transverse section of leaf-segment of *Kobinia Prunoides*, showing the absence of a foliar ray.

Fig. 25. Transverse section of the foliar ray in the woody herb *Meibomia oiba*.

PLATE XIII.

Fig. 26. Total transverse section of the nodal region of the woody herb *Gerardia*, showing the presence of two foliar rays corresponding to the two opposite leaves. The ray on the lower side has just begun to appear.

Fig. 27. Transverse section of the foliar ray shown in the upper region of Fig. 26, more highly magnified.

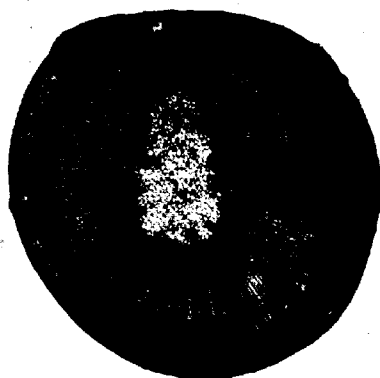
Fig. 28. Transverse section of the foliar ray in *Solidago canadensis*, from the base of the aerial axis.

Fig. 29. Tangential section of the foliar ray of *S. canadensis*, from the thicker region of the aerial stem.

Fig. 30. Radial section of the foliar ray from the woody region of the aerial stem of *S. canadensis*.

Fig. 31. Tangential section of the foliar ray of the lower region of the aerial axis of *Helianthus tuberosus*.

Fig. 32. Tangential section from a lower aerial node of *Helianthus annuus*, showing the organization of the foliar ray. The magnification is the same as in Fig. 31.



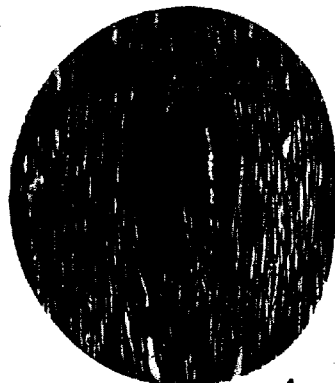
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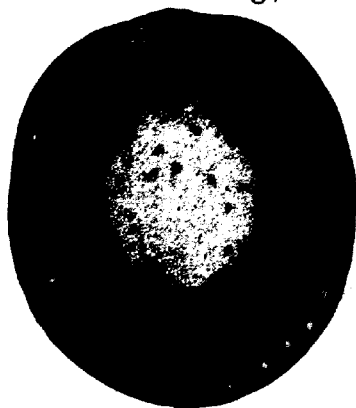
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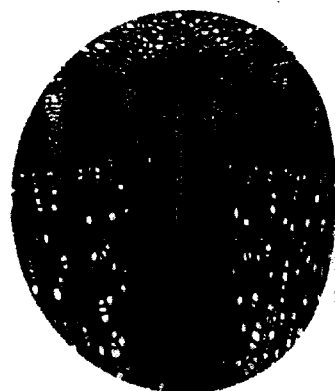
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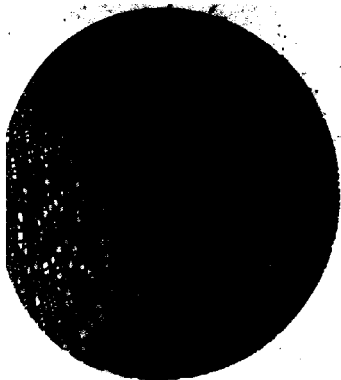
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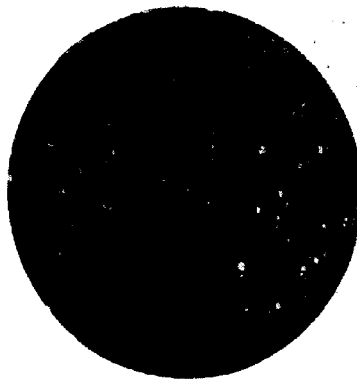
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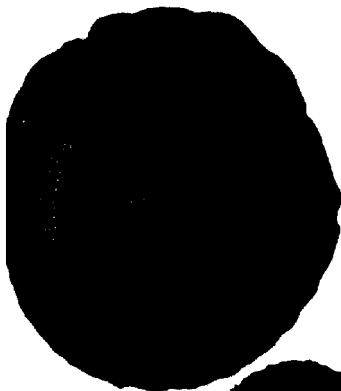
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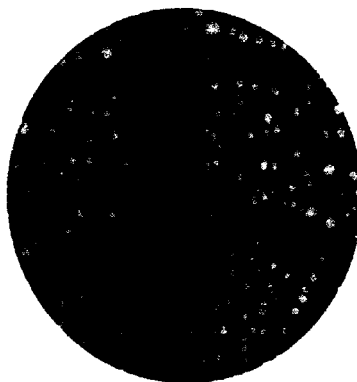
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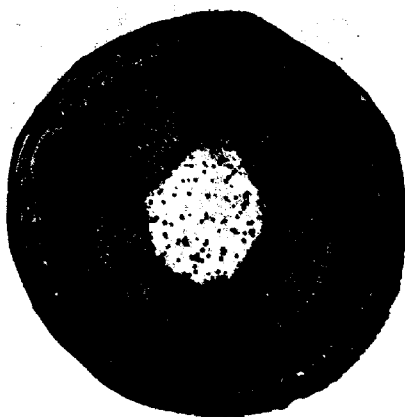


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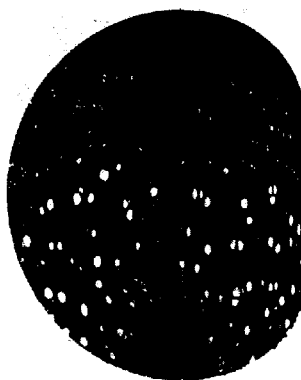


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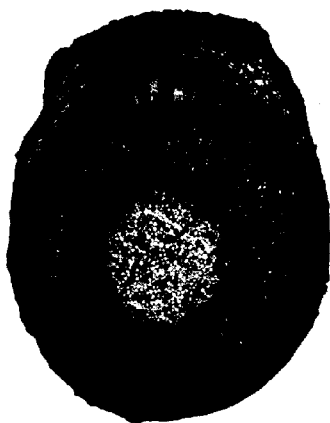
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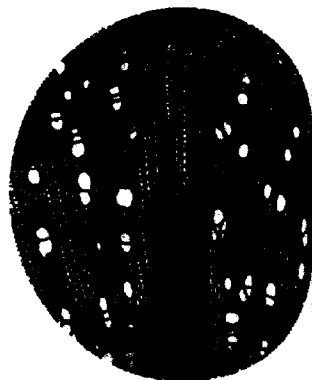
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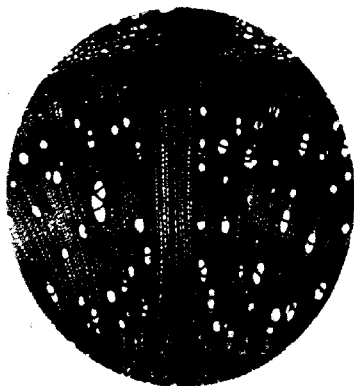
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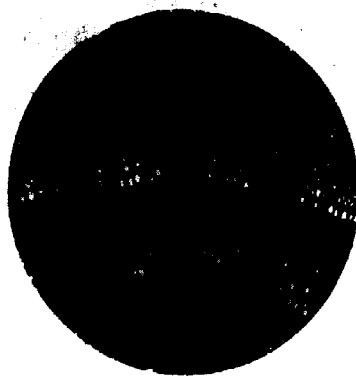
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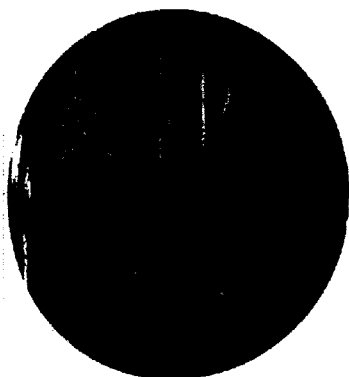
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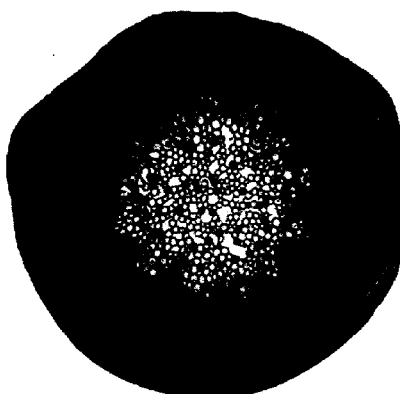
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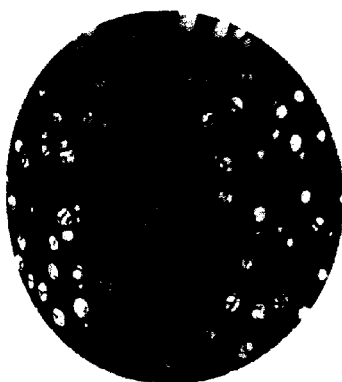
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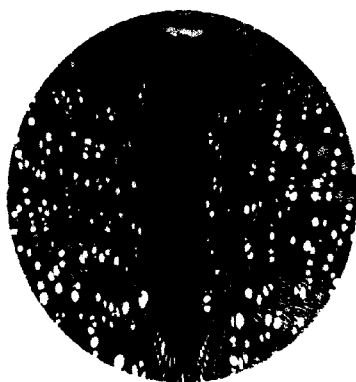
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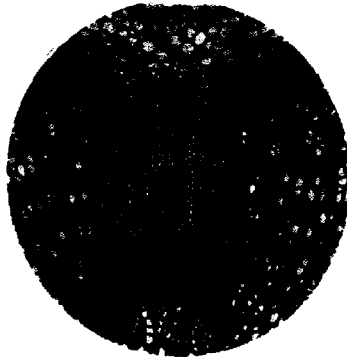


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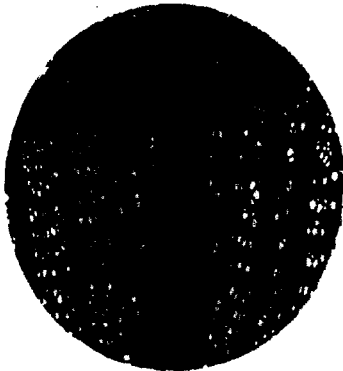
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A Contribution to the Study of Water-conductivity in Sycamore Wood.

BY

M. G. HOLMES, B.Sc.

With thirteen Figures in the Text.

THE observations which form the subject of the present paper are based on an anatomical investigation of young Sycamore plants, *Acer Pseudoplatanus*; they have special reference to the number, size, and distribution of the water-conducting elements in the wood, considered in its transverse aspect, at intervals throughout the stem.

MATERIAL.

The work was carried out on some Sycamore saplings, collected in the dormant condition in February, 1919, the plants being from two to five years old. The lengths of these shoots are indicated diagrammatically in Fig. 1. They are divided into segments, each corresponding with the growth in length during one season, and the segments are lettered *a, b, c* . . . from the base of the plant to its apex. Within each segment the internodes are numbered 1, 2, 3 . . . from the base of the segment upwards. It will be seen that the specimens S2 and S4 show three years' growth, and S3 five years', the growth being continued each spring by the development of the apical bud. S5 shows two years' growth only; the bud at the apex of the main stem, *a*, failed to develop in 1918, and the laterals reached a considerable length; these are lettered *b, c, d* . . . The development of branches from the buds formed in the axils of the pairs of leaves at the nodes is more vigorous in the upper part of the segment, and as a rule the shoot formed from the apical bud is by far the strongest of its season. These specimens are comparable in length with the stool shoots of Hazel and Ash, previously described (6 and 7), but the latter were of one season's growth only, and investigation was confined to wood of the first year. The Sycamore specimens show a comparatively short growth in length to have taken place during one season, with comparatively few nodes, and in dealing with them it has been necessary to take into account wood of several years.

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ANATOMY.

In a transverse section of the internode of the Sycamore stem, the wood has a fairly uniform appearance. At the edge of the pith, characteristically six-sided in shape, are six conspicuous leaf-trace bundles, containing

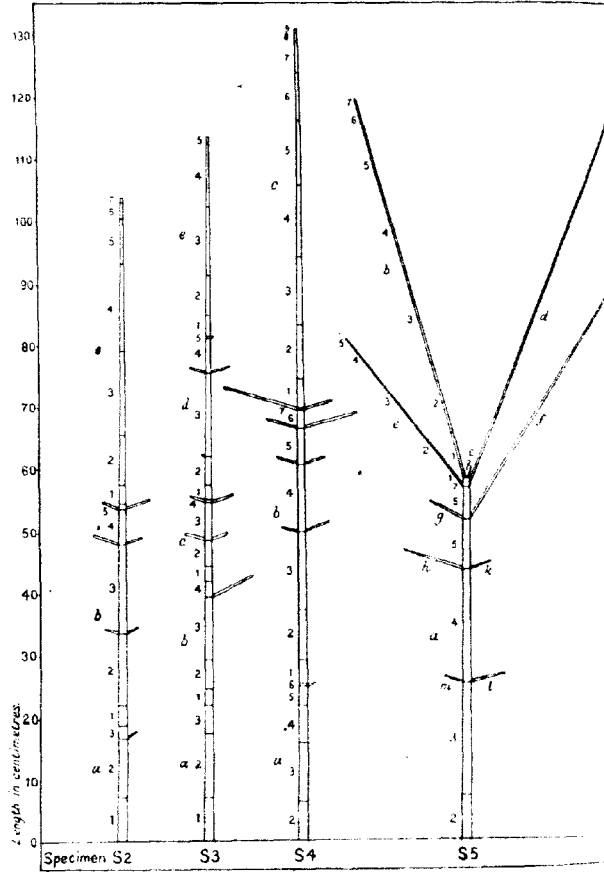


FIG. 1.

spiral vessels, with smaller bundles between these. The bundles consist of several rows of vessels close together, separated from the thin-walled empty pith cells by a layer of thicker-walled parenchyma filled with starch. In the smallest sections there is little else in the xylem beyond these bundles, which are separated by medullary rays several rows of cells in width. These primary rays are distinguishable in the larger sections, traversing the

rest of the ring of wood, with numerous smaller rays between. The typical xylem has a basis of wood fibres, interspersed fairly evenly with vessels, the tracheides and wood parenchyma being less conspicuous. Where the shoot is more than one year old the annual rings of wood are easily distinguished by the contrast in size and character between the elements on either side of

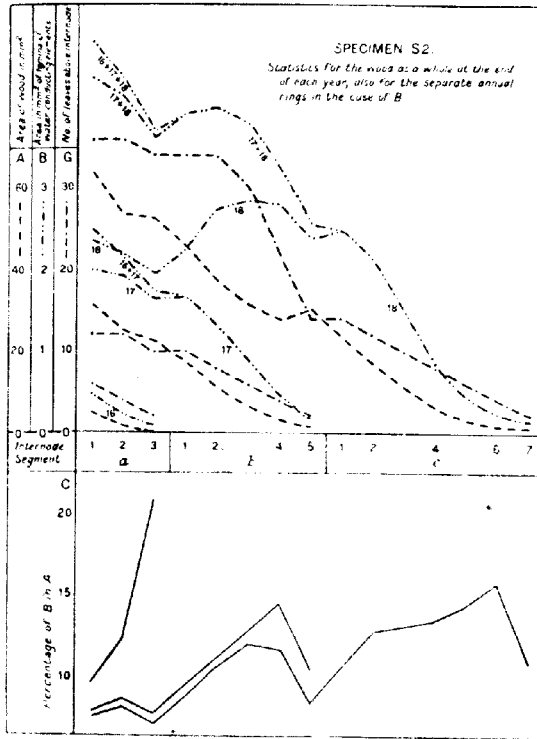


FIG. 2.

In this, and in the figures which follow, the identification-marks of the different curves will be found in the vertical columns A, B, C, &c.

the line. The abundance of starch is a striking feature of the wood in the winter condition.

The water-conducting elements are mostly of the nature of vessels; these have short wide segments, communicating by a single large round hole in each oblique dividing wall; the rest of the wall is thickly covered with bordered pits, and strengthened internally with fine spiral bands. Typical spiral vessels occur next to the pith, followed by somewhat transitional elements with elongated pits. Next to the outer limit of the annual ring

there occur at intervals radial rows of smaller water-conducting elements; most of these are perforated in their end walls, but the outermost are of the nature of tracheides, very similar in shape to the wood fibres. They have pits and spiral bands as in the vessels, and are well supplied with bordered pits on their tangential walls. At the limit of the year's growth the last tracheides frequently come into contact with the first vessels of the following year, and serve apparently to maintain a connexion with the latter, as stated by Strasburger (1, p. 216).

The mechanical function of the wood is served by the presence of a preponderating number of wood fibres, long pointed cells, empty, the

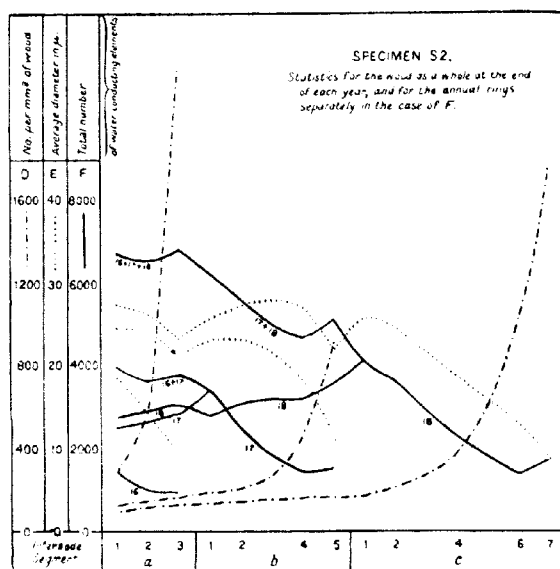


FIG. 3.

walls sparingly supplied with simple oblique pits. Surrounding each vessel is a zone of greater or less extent consisting of cells filled with starch grains, but otherwise closely resembling the empty fibres, as seen in both transverse and longitudinal section. They have simple oblique pits, chiefly on the radial walls, and their walls may be slightly thicker and more lignified than those of the neighbouring fibres. Strasburger (1, p. 215) distinguishes these elements as dead and living wood fibres. At the end of each annual ring there is a layer, several cells deep, between the rays consisting almost entirely of living, starch-bearing fibres, and radial rows of vessels and tracheides. The cells decrease in size outwards in each annual ring, and become thicker walled and flattened towards this limit, which

is thus easily identified. Most of the starch, apart from that in the ray cells, is stored in these prosenchymatous elements, and wood parenchyma is only sparingly developed, rows of shorter, more abundantly pitted, starch-bearing cells occurring occasionally near the vessels and among the flattened cells of the outer part of the annual ring. The rays, from one to six cells wide, may be anything up to forty cells high (1, p. 217); where ray or wood parenchyma cells come into contact with vessels, the dividing walls have numerous half-bordered pits, and these and the living wood fibres evidently form one continuous storage system. In the oldest parts of

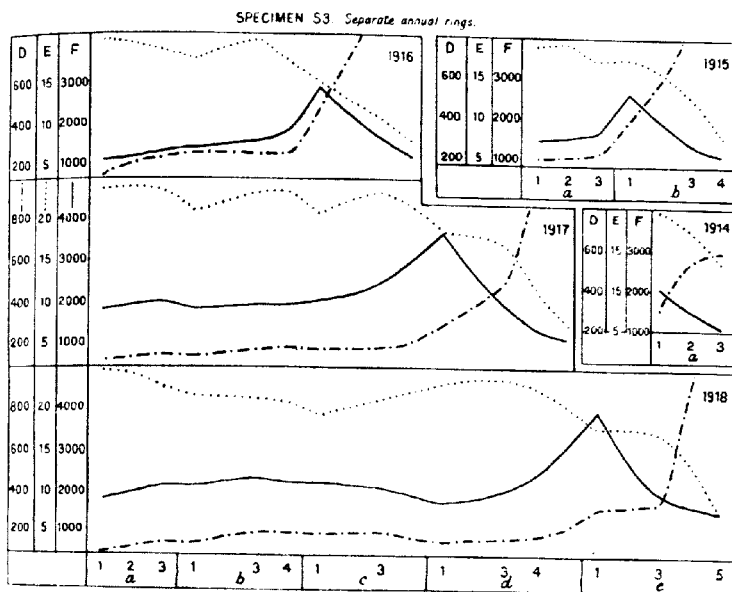


FIG. 4.

the wood examined, five years', there is abundant starch in the storage cells. According to Haberlandt (3, p. 684), *Acer* is one of the few deciduous trees which have no heart-wood; he states also that, generally, in normal circumstances, only the outermost annual rings of a shoot serve to convey the transpiration current, the more internal portions of the sap-wood serving for the storage of water and synthetic products.

The relatively small number of vessels and prevalence of fibres in Sycamore wood is mentioned by Strasburger (1, p. 216); and he states that the comparatively small width of the vessels, averaging about 0.035 mm., is remarkable in view of their low proportion. Solereder (2, p. 271) also notices the small size of the vessels in Sycamore, and gives the diameter of

the lumen as 0.06 mm. In the present paper variation in the average diameter of the vessels for different shoots and different parts of the same shoot will be illustrated, but at this point it may be mentioned that the extreme range of individual diameter among the measurements made for these young plants is 57 to 3 μ .

METHOD.

In working out statistics for the size and proportion of the water-conducting elements in the wood, a method similar to that previously described for Hazel and Ash has been followed (6 and 7), the data being

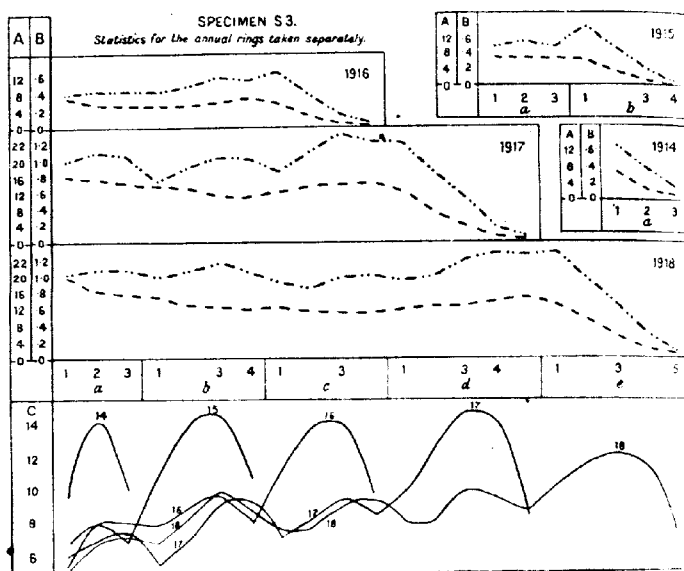


FIG. 5.

obtained from transverse sections of the wood taken at the middle points of the selected internodes. The figures are only approximate, of course, and contain no information as to the longitudinal characters of the elements, or the resistance at the nodes. In all cases graphs were drawn from these figures to be comparable with those constructed for Hazel and Ash; the same vertical scales were taken for all, while the horizontal scale for the Sycamore is the same as that for the Ash, and double that for the Hazel, to correspond with the number of leaves borne at each node.

Here it is necessary to explain that a mistake appears in the results given in the Hazel and Ash papers, which the writer much regrets. The figures for B were worked out on the basis of a formula in which the squaring

was done in the wrong place, so that they are in all cases rather too low. This makes the figures for C also too low, but the remaining figures are not affected. Thus, while retaining a similar general shape, but not exactly the same, the curves for B and C in all the diagrams ought to be higher. The numbers given to represent the limits in variation in the values for C (7, p. 263) should be as follows:

Ash 2.32 to 12.25 % (instead of 1.68 to 9.5)
Hazel 4.09 to 25.4 % (instead of 3.21 to 20.26)

Further corrected figures will be given below, for comparison with those obtained for Sycamore. This correction does not, however, materially affect the general conclusions of the earlier papers.¹

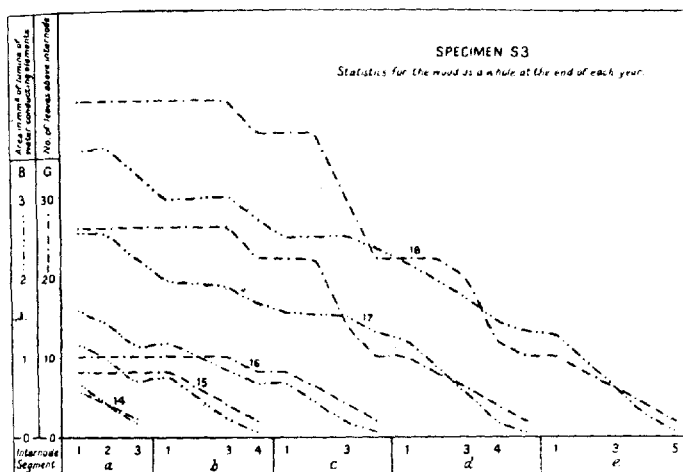


FIG. 6.

Returning to the Sycamore, it will be obvious that, when it comes to any part of the stem which is more than one year old, equal intervals on the base line do not any longer correspond with equal numbers of leaves supplied; some nodes are bare, while others have one or two lateral

¹ The incorrect formula was based on the corresponding values for E and F , as follows:

$$B = F \times \pi \left(\frac{f}{2}\right)^2 \div 10^6 \text{ mm}^2 \quad F \text{ being in } \mu^2$$

² was worked out from the value found for B .

The correct calculation is as follows:

Suppose that the diameters are measured at intervals of $\epsilon \mu$, and let $F = (f_1 + f_2 + f_3 + \dots)$, in which f_1, f_2, f_3, \dots represent the numbers of elements having diameters of $1\epsilon, 2\epsilon, 3\epsilon, \dots \mu$; then

$$B = f_1 \pi \left(\frac{\epsilon}{2}\right)^2 + f_2 \pi \left(\frac{2\epsilon}{2}\right)^2 + f_3 \pi \left(\frac{3\epsilon}{2}\right)^2 + \dots \mu^2 \\ = \frac{\pi \epsilon^2}{4 \times 10^4} (f_1 + 4^2 f_2 + 9^2 f_3 + \dots) \text{ mm}^2.$$

branches, of various lengths. This circumstance introduces irregularities into the curves, beyond those due to differences in the sizes of the leaves. About the latter no information can be given for these specimens; but to represent the number of leaves borne along the shoot during each season, another line, G, has been introduced into some of the graphs; the height of this line at the position representing each internode indicates the number of leaves on the stem above that internode.

In all cases where the stem cut was more than one year old, the data were worked out for each annual ring separately, and the figures were also combined to furnish statistics representing the condition of the wood as a whole at the end of each year's growth. From these figures it is

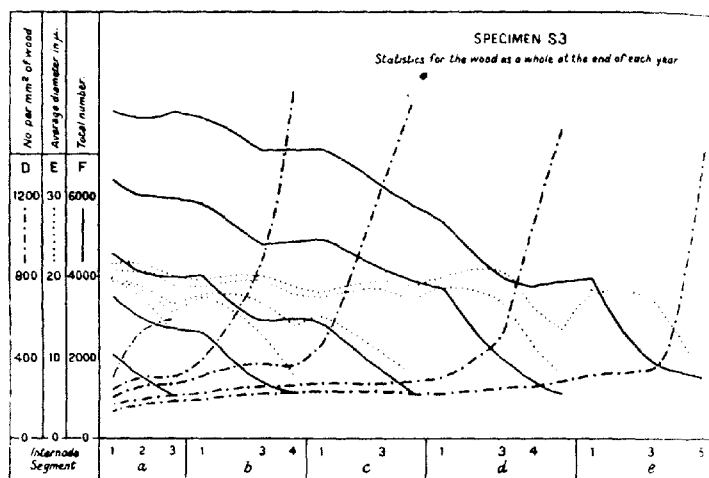


FIG. 7.

possible to draw a line representing quantitatively the extent of development of a particular character, for a particular annual ring as far as it extends along the stem, or for the whole of the wood present at the end of a particular year. Considering the curves which come into the latter category, it is evident that those representing the areas of the wood along the same stem at the end of each year of its growth, A, or the areas of the lumina of all the water-conducting elements, B, or the numbers of these elements, F, may be drawn without interference between the same co-ordinates; this is sufficiently true in practice of the other curves also, representing specific rather than absolute data. But the curves which belong to the former category may interfere in this sense, so that separate diagrams to illustrate these have been introduced in some cases. Thus for specimen S₃, which is five years old, there are five lines for each of the seven

characters A to G, in each category; the thirty lines A to F of the first category are set out in Figs. 4 and 5, while the thirty-five lines A to G of the second group appear in Figs. 6, 7, and 8. There is, of course, only one set of G lines, and each pair of corresponding lines from the two groups has a common ending in the one-year part of its course. In the cases of S2 and S4, each three-year-old specimens, the twenty-one lines have been drawn for the wood as a whole at the end of each year, in Figs. 2 and 3, and Figs. 9 and 10. The other lines are not given, except for B and F, and these additional portions, giving values for the annual rings taken separately, are inserted into the same diagrams for comparison. In the case of S5, the statistics for three of the one-year-old laterals are indicated in Fig. 11, while the lines for the main stem of the whole two-year plant, ending in the uppermost, and very unequal, pair of branches taken together as a leader,

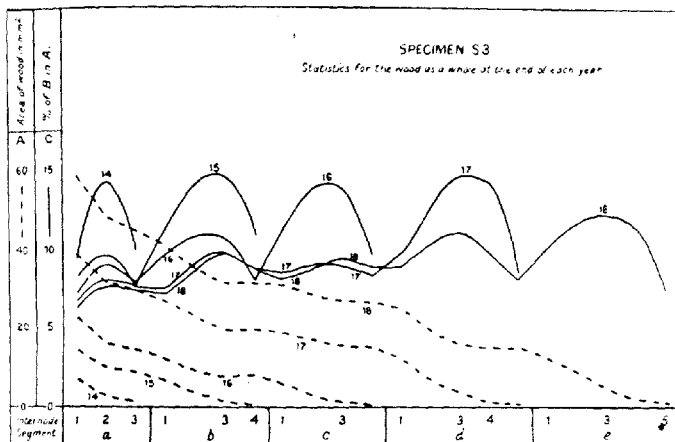


FIG. 8.

are given in Figs. 12 and 13. These include the lines of both categories: they are distinguished by the insertion of dates, the numbers referring to the years in which the wood was developed.

RESULTS.

It will be convenient, in discussing the significance of these observations, to begin with the simpler parts which have reference to first-year wood, and afterwards to consider the whole series in the two categories mentioned above.

Wood of the first year. It will be seen that a general similarity in direction exists in the curves constructed for first-year wood in the three plants. Curve A, giving in sq. mm. the area of the wood in transverse section at the middle of each internode, shows a gradual decline from base to

apex in each first-year segment, steeper as usual towards its beginning. It will be seen from Figs. 1, 2, 5, and 10 that in S₃ the annual additions to the plant were shorter and thinner than in S₂ and S₄. The water-conducting area in this wood is indicated in Curve B, which gives the total area in mm.² of the lumina of all the water-conducting elements in the transverse section of the wood at each internode. This also shows a simple decline from the base to the apex of each shoot in its first year, somewhat flatter towards the end; it corresponds to the simple successive decreases in the number of leaves supplied, the upper ones being smaller than the lower ones. The total number of the water-conducting elements in each section, as shown in

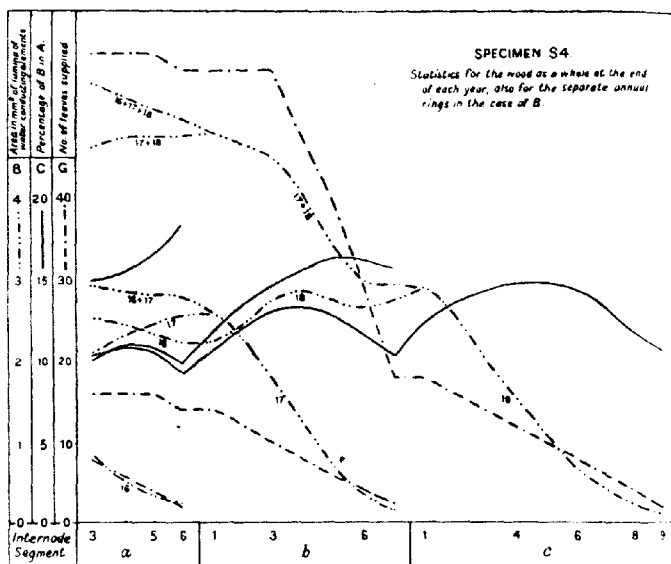


FIG. 9.

Curve F, reveals again a decline from base to apex, but there is a tendency towards a marked flattening, or even a rise, quite close to the end, Figs. 3, 4, 10, and 11. These elements, however, are particularly small, as shown by the steeper decline of Curve E, which gives the average diameters of their lumina in μ , towards the end of each first-year shoot. The vessels of large diameter occur in the lower part of the shoot, where the average diameter falls quite slowly, while they are absent from the apical part. The distribution of the conducting elements may be inferred to some extent from Curve D, which shows the number occurring per square millimetre in the transverse section of the wood in each internode; the line rises gradually at first, and then with increasing rapidity towards the end in each first-year

shoot, Figs. 3, 7, 10, and 11. The shape of Curve D in Sycamore is much more like that characteristic of Hazel than that of Ash stool shoots, where the wood is particularly poor in vessels. Considering only wood of the first year, the limits of variation in the width and distribution of these elements, among the measurements made for the three plants, may be compared as follows :

	<i>Ash.</i>	<i>Sycamore.</i>	<i>Hazel.</i>
Range in values of B in μ	27.85 to 10.14	27.85 to 7	23.27 to 4.9
" " actual diameters in μ	80 " 3	60 " 3	148 " 2
" " values for D	32 " 633	137 " 2233	115 " 4000

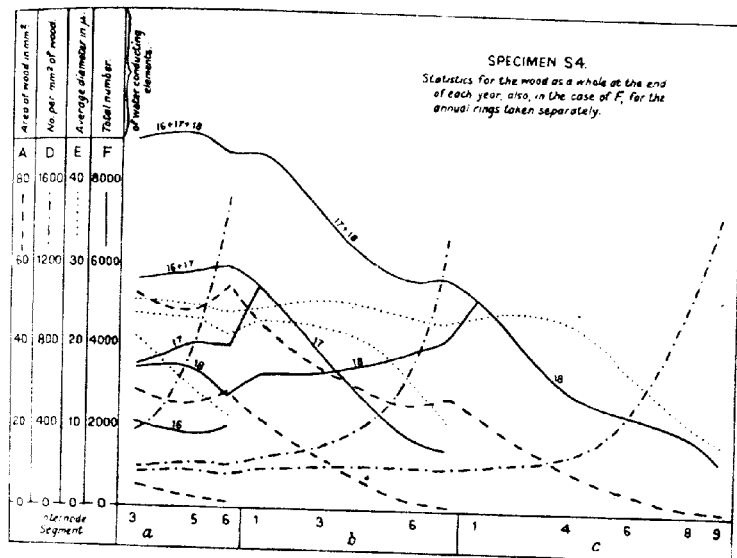


FIG. 10.

The result is that the specific conductivity for the first-year Sycamore wood, as represented by Curve C, which gives the percentage of B in A, is near that for Hazel, both being higher than that for Ash :

	<i>Ash.</i>	<i>Sycamore.</i>	<i>Hazel.</i>
Range in values of C, %	2.32 to 12.25	7.19 to 20.93	4.09 to 25.4

It must be remembered that this shorter range of values for Sycamore as compared with Hazel has been obtained from shorter shoots, not altogether comparable with stool shoots. A particularly low specific conductivity at the base is characteristic of stool shoots, as mentioned by Farmer (5, p. 241). It is apparent from Figs. 2, 8, 9, 11, and 12 that there is considerable variation in the shape of Curve C, as among the different specimens, though there is a tendency to a fairly close similarity in its shape

for the successive first-year segments of the same specimen. In general, there appears to be a similar tendency towards a rise followed by a fall, as was shown for Hazel and Ash. That is, there is a preponderance of fibrous elements towards the base of the shoot, where mechanical efficiency is most necessary, while at the apex the specific conductivity is again reduced owing to the small size of the conducting elements, in spite of their large number. Exceptions to this occurrence of rise and fall in Curve C for first-year shoots are shown in S2a, 1916, a short basal main stem, in which there is a rise only; and in S5c, a very weak lateral in which there is only a fall, cf. A8e.

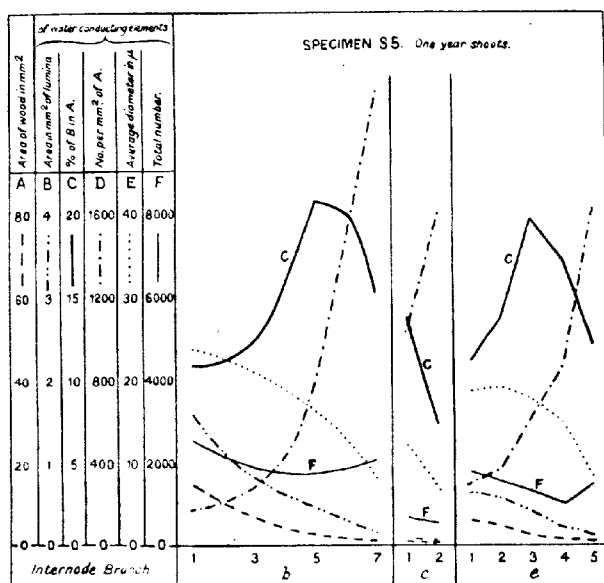


FIG. 11.

Wood of the second and following years. We come now to the consideration of the character of the wood in parts of the stem more than one year old, apart from that composing the innermost annual ring. In the second and later annual rings leaf-trace bundles are absent, so that the wood has a more uniform structure; the vessels also reach a greater diameter. The differences between the wood of the first and subsequent years are illustrated in Figs. 4 and 5, in which lines, representing the characters of each annual ring separately, are drawn for S3, the five-year-old specimen. The transverse sectional area of the wood composing each annual ring, Curve A, Fig. 5, is maintained at a fairly constant level in the internodes as its course

is traced downwards, this level being about the same as that at the first internode of the final segment. The same condition may be inferred from Figs. 2 and 10; here the rise in the curve at the final internodes of the lower segments is due to the fact that the wood widens out at the nodes, and that these internodes are very short; see Fig. 1. The size and numbers of the water-conducting elements in these annual rings are illustrated in Fig. 4. Curve F shows a maximum for the total number at the first internode of the final segment, and a general tendency to decrease in the number, as the curve is traced backwards. This may be seen also in Figs. 3 and 10. The number of these elements per unit area naturally tends to decrease downwards also, as appears from Curves D, Fig. 4; the special provision of mechanical elements at the base of the plant, as mentioned by Farmer (5, p. 241), is indicated by a comparison of Curves A and D, Figs. 5 and 4, in this region. With the general decrease in number downwards is associated to some extent an increase in average width, as shown in Curves E, Fig. 4. The influence of the presence in the second and outer annual rings of wider vessels than occur in the innermost ring is apparent, and there is a slight increase downwards and outwards apart from this. The following table will make this clearer, from the point of view of maximum diameters:-

Specimen.	Max. diam. of vessels in μ .			Specimen.	Max. diam. of vessels in μ .		
	Annual ring of	First-year segment.	Lower segments.		Annual ring of	First-year segment.	Lower segments.
S 2	'16	50	—	S 4	'16	40	—
	'17	50	57		'17	53	60
	'18	50	67		'18	56	67
S 3	'14	38	—	S 5a	'17	60	—
	'15	46	46		'18	—	66
	'16	40	47	S 5b	—	60	—
	'17	50	53	S 5c	—	56	—
	'18	50	57	S 5d	—	57	—
				S 5e	—	48	—

Among the figures obtained for average diameters and numbers per unit area, in wood of the second and outer rings only, taken separately, the limits of variation are as follows:

Range of values for E, in μ	31.32 to 17.04
" " " D	85 " 147

These various factors contribute to keep the conducting area, as represented by Curves B, Fig. 5, fairly level along its course in each separate annual ring, with a maximum generally at the first internode of the last segment. This appears also in Fig. 9, but is less clear in Figs. 2 and 12. Of course Curve B represents the absolute conducting capacity in its transverse aspect only; its fall backwards from the maximum is probably compensated, and more than compensated, by the greater length and decreased

resistance of the wider vessels. The final result in this series, that is, the specific conductivity in its transverse aspect, is indicated in Curves C. Fig. 5. The percentage of B in A in each separate annual ring shows less variation as the line is traced backwards; there is a general tendency towards a lower specific conductivity in the lower part of the stem. It is sufficiently evident from the diagrams that the maximum values for C occur

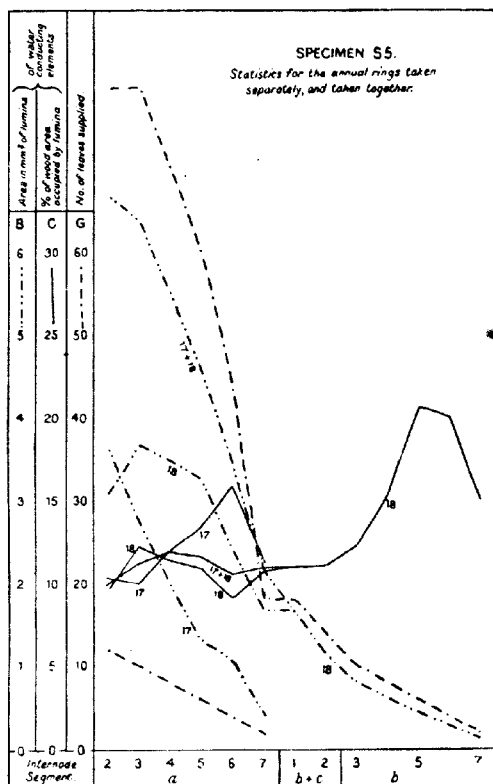


FIG. 12.

in first-year wood. The range of values observed for C in the wood of separate annual rings, apart from first-year wood, is 5.05 to 12.42 per cent.

Statistics for the wood as a whole. Professor Farmer's figures for specific conductivity (5) for shoots of various ages are worked out on the basis of the sectional area of the whole of the wood present. With these may be compared as far as practicable the values plotted out in Figs. 2, 3, 6, 7, 8, 9, 10, 12, and 13, to indicate the condition of each factor at the end

of each growing season. For instance, the repetitions of Curve A in Figs. 2, 8, 10, and 13 give a sufficiently clear idea of the general effect of the yearly additions to the area of the wood. The curves are fairly smooth, apart from the local rises at the ends of segments, as noticed above. Turning to the statistics for the water-conducting elements as given in Figs. 3, 7, and 10, we find that the curves for D become smoother and flatter, as the influence of the first-year wood is less felt. On plotting the total numbers of these elements present in the whole of the wood, there is produced for each plant a series of descending curves, F, in which the most con-

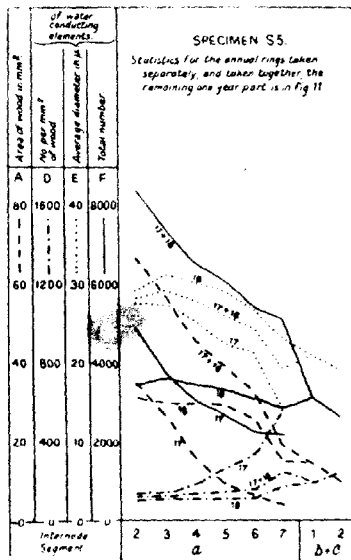


FIG. 13.

spicuous irregularities are local maxima at the ends of the segments. In some cases these are sufficient to eliminate the effect of the maxima at the beginnings of the segments, which appear in Curves F for the annual rings taken separately, as pointed out in Fig. 4; examination of Figs. 3 and 10 will serve to illustrate this point. At the end internodes, naturally, F includes many very small elements, as is indicated by the local minima in Curves E, Figs. 3, 7, and 10; consequently in Curves B, Figs. 2, 6, and 9, which give the result of taking these two factors together, there is less irregularity. If Curve B be taken to express as far as possible the absolute conductivity of the wood as a whole, its general slope downwards from base to apex of the plant may be considered with reference to the number of leaves borne

on the plant. As explained above, it is only in the final segment of the line that the number of leaves supplied decreases regularly in correspondence with equal intervals on the base line; consequently it is to be expected that in passing through the remaining segments Curve B will show less regularity. In Figs. 2, 6, 9, and 12, an attempt is made to correlate the shape of Curve B with the number of leaves supplied, as represented by the line G. It is necessary, of course, to fix an arbitrary vertical scale for G, as data for the actual areas of the leaf surfaces are not available. The line should be examined in connexion with Fig. 1. From observations on plants in leaf, it is clear that the leaves on the lower part of the leader are larger than those on its upper part, and on the weaker branches. Thus, taking as an example the line G for S4 in 1918, Fig. 9, if G were to represent leaf area instead of leaf number, the part of the line between a_3 and b_6 would be lower and that between b_7 and c_5 would be higher. It is to be noticed that the uppermost pair of laterals are attached to the node between internodes 6 and 7 of segment b , so that b_7 and c_1 serve the same number of leaves during 1918; and that the horizontal piece of Curve G is reflected in Curve B. The flattening from b_3 towards the base of the plant is evident in Curve B, but less well marked. In general, Curve G seems to agree best with the corresponding Curve B for the wood as a whole. Of course, other factors, such as the length of the vessels and the proportion of wide to narrow vessels, ought also to be taken into account, and this might alter the relation. Salisbury (4) has drawn attention to the close correlation so far observed between leaf function and the amount and constitution of the xylem in the petiole, and there is at any rate reason to believe in the existence of such a correlation extending into the balance between the annual rings of wood in the stem.

General comparison. It is in particular Curve C, for the wood as a whole, which should be compared with Professor Farmer's figures for specific conductivity (5). The comparison cannot be altogether satisfactory; in Professor Farmer's experiments a standard length of shoot, 15 cm., was used, regardless of the number of nodes; and the specific conductivity was worked out on the basis of the area of the transverse section of the wood at the middle of this length. In the present paper, length is disregarded in favour of the number of internodes, that is, the number of leaves supplied; and it is shown that there is a considerable variation in the value arrived at for specific conductivity at different parts of the same shoot, and within a length of 15 cm. This variation, again, applies only to values for internodes, and neglects conditions occurring at the nodes, while it takes into account only the transverse aspect. Thus it is difficult, from the figures obtained in this inquiry, to make general averages, which will be comparable with Professor Farmer's figures. An average can be obtained for each shoot at the end of each year, from the values of C for every internode along

the shoot, and this is the information given in the following table, which includes also corrected figures for the Hazel and Ash shoots on the same basis. In this way, however, the shorter internodes have more weight in determining the averages, when compared with averages taken from values for equal lengths. From such considerations it is evident that no very close correlation can be expected between the two sets of figures.

	Hazel.		Ash.		Sycamore.	
	Specimen.	Average of values for C.	Specimen.	Average of values for C.	Specimen.	Average of values for C.
Separate averages.	H 7	10.264	A 3	4.596	S 2 { at end of '17	10.354
	H 8	10.296	A 4	4.112	" " '18	11.014
	H 9	11.712	A 6	7.722	S 3 { " " '15	10.857
	H 10	14.512			" " '16	10.095
Total		46.784		16.43	S 4 { " " '17	12.523
					" " '18	11.94
					S 5 { " " '18	12.849
General average		11.696		5.476		79.632
						11.376
Farmer's figures for specific conductivity.	Stool shoots	31 ± 9 (5, p. 248)	Stool shoots	14 ± 10 (5, p. 248)	Young trees, 2 and 3 years old	34.63 ± 3.3 (5, p. 239)

The averages given in the above table were selected to give the fairest comparison with the figures quoted in the last line. The remaining averages, not included above, are given in the table which follows:

Ash.		Sycamore.	
Specimen.	Average for C.	Specimen.	Average for C.
A 8a	4.544	S 5b	15.008
A 8b	6.59	S 5c	10.45
A 8c	7.5	S 5e	14.656
		S 2, at end of '16	14.343
		S 4, " " '16	14.838
		S 5, " " '17	11.56
		S 3 { at end of '14	11.203
		" " '17	9.803
		" " '18	9.098

SUMMARY.

In this paper are described the results of an investigation into the constitution of the wood of young Sycamore plants, with special reference to its efficiency for the conduction of water. The work is a continuation of that begun on stool shoots of Hazel and Ash, but the observations are not confined to first-year wood. The writer takes this opportunity of correcting a mistake which appears in some of the figures given in the earlier papers. With regard to wood of the second and outer annual rings, it is pointed out that there is less variation in the specific conductivity for water than in the first-year wood: the vessels are wider, but less abundant, and this tends to make the figures for specific conductivity become somewhat lower. In general the specific conductivity in the wood of young Sycamore plants, estimated in its transverse aspect, is near that found for Hazel stool shoots, and higher than that for Ash.

NOTE.

Miss Rivett has been so good as to let me see a copy of her paper (8) on *Rhododendron* and *Holly*, before its publication. On p. 549 she draws attention to the lack of consistency between the deciduous and evergreen woods investigated, in comparing the data for C with Professor Farmer's figures for specific conductivity. The correction mentioned above does not increase the values of C for *Hazel* and *Ash* sufficiently to bring them into a relation with the corresponding specific conductivity values, similar to that which obtains in the case of *Rhododendron* and *Holly*. This will be seen from the following table giving mean average values in all cases:

<i>Plant.</i>	<i>C.</i>	<i>Spec. cond.</i>
<i>Rhododendron</i>	23.8 %	16
<i>Holly</i>	8.0 %	9
<i>Hazel</i>	11.7 %	31
<i>Ash</i>	5.5 %	14
<i>Sycamore</i>	11.4 %	35

It appears, therefore, that the anomaly must be due to the incidence of longitudinal characters. Professor Farmer has pointed out (5, p. 249) the effect, in lowering the specific conductivity, of the short vessels which are characteristic of the evergreens. Miss Rivett concludes that the narrower and probably shorter vessels in the evergreens offer more resistance to the passage of water, so that for them a comparatively high value for C is to be expected. In this sense a particularly low value for C should be found in the case of *Ash*, while that for *Sycamore* should be somewhat lower than that for *Hazel*.

I am glad to take this opportunity of thanking Professor Farmer for the provision of material and for his help during the progress of the work.

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Observations on some Physical Properties of Protoplasm by Aid of Microdissection.¹

BY

WILLIAM SEIFRIZ.

With one Figure in the Text.

INTRODUCTION.

EARLY workers on protoplasm dealt entirely with living material. The advent of cytological technique with its methods of fixing and staining opened up a new and fertile field of investigation, leading to the discovery of many heretofore hidden structures of the cell. But it was soon realized that many of these structures were the direct result of the fixing and staining of the material. Consequently, there is to-day a pronounced reaction against this method of making observations and an increasing sentiment in favour of observations on living material.

METHOD.

Morphological work on living protoplasm has been greatly stimulated by the recent introduction of an ingenious technique generally known as the microdissection method. The instrument used in this method is a modification of the Barber (2) pipette-holder and consists of two mechanical needle-holders, each capable of being moved in three directions. Glass needles with very sharp and rigid tips are used for dissecting the material, which is suspended in a water film on the under side of a cover-glass that constitutes the cover of a small moist-chamber under the microscope objective. A complete account of the method and the technique connected with it is given in an article by Chambers (10), to which the reader is referred.

PRECAUTIONS.

The student of living protoplasm soon learns to appreciate the necessity of knowing as far as possible the exact condition of the material which he is studying. Indeed, the great problem in microdissection is to know when one is observing living, and when dying or dead protoplasm. While the structure and behaviour of dead protoplasm is in many instances highly

¹ Botanical Contribution from the Johns Hopkins University, No. 68.

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instructive, yet they differ, often markedly, from the structure and behaviour of living protoplasm. However, where a structure cannot be readily seen in the living condition, but does, because of some physical or chemical change, become clear when dead, we are not, therefore, justified in utterly disregarding the evidence based on dead material. For example, the presence of a protoplasmic membrane in the living state cannot be indubitably established, yet the presence of the dead plasma-membrane is in some instances strikingly evident. As evidence for support of the existence of a living membrane, the presence of a dead membrane is not to be altogether ignored.

Not only must every precaution be exercised to avoid describing living protoplasm from observations on dead protoplasm, but one must also take care to ascertain if the substance under observation is true protoplasm or a modified form or a product of it, such as vacuolar sap or yolk, for example. Many plant cells are more than three-fourths sap with only a layer of protoplasm lining the cell-wall, and the volume of some fish eggs is nineteen-twentieths yolk with but a thin coating of protoplasm enveloping the latter (before fertilization). In working on such material it is difficult to be certain that one is observing the behaviour of true protoplasm and not of sap or yolk.¹

TERMINOLOGY.

While some writers condemn too precise a definition of a word it is nevertheless true that a very broad use of a term leaves open the possibility of a great number of interpretations, none of which may coincide with the specific one in the mind of the writer. The word 'membrane', because of its free and lax use, is an excellent example of the confusion which exists in biological nomenclature. The following are some of the expressions which have been used to refer to a single cell structure, namely the plasma-membrane: 'phase boundary', 'surface film', 'surface layer', 'ectoplast', '(vacuole) wall', 'ectosarc', and 'hyaloplasm'. Where the jumble is one so hopeless as this, it is often convenient to use any one of the expressions in a purely abstract sense. This is what Stiles and Jörgensen have done. They (41, p. 533) have recently put in a 'plea for definiteness of statement and for the avoidance of semi-mystical expressions such as "permeability" or "plasma-membrane"'.¹ The plea for definiteness is a worthy one. But their solution of the difficulty which the many expressions for the protoplasmic membrane present, by using 'plasma-membrane' to mean 'that part of the cell which is concerned in the phenomenon of permeability without reference to its actual location in the cell' (40, p. 50), is, perhaps, permissible where one has no interest in the plasma-membrane as such; it does not, however, solve the problem of the existence

¹ Some other precautions in technique are given in a recent article on Viscosity values of Protoplasm (38).

or non-existence of a plasma-membrane. To one concerned in establishing the possible morphological identity of a protoplasmic membrane there is but one course, namely, accurately to describe the thing and let this description stand as a definition for the name used.

MATERIAL.

The data upon which the following discussions are based were obtained by the study of a considerable variety of material. While the conclusions reached may be regarded as pretty generally applicable, it is to be understood that the statements made refer only to the organism under discussion at the time. Some of the physical properties of protoplasm are characteristic of *all* protoplasm, yet prominent dissimilarities do occur between even closely related species.

The following types are the chief ones used for this study: the myxomycetes *Ceratiomyxa*, *Badhamia*, *Arcyria*, *Cribraria*, and *Fuligo*; the ova of the rock-weed *Fucus*; the freshwater algae *Spirogyra* and *Vaucheria*; *Eldodea*; the bread-moulds *Rhizopus* and *Zygorhynchus*; pollen tubes of the Blue Flag *Iris versicolor*, of the Beach-pea *Lathyrus maritimus*, and of the Dog's-tooth Violet *Erythronium revolutum*; the protozoa *Amoeba* and *Euplotes*; the ova of the sand-dollar *Echinarachnius*, of the sea-urchin *Tripleneustes esculentus*, and of the silver (English) hake *Merluccius*.

The experimental work on these forms was done mostly in the Botanical Laboratory of the Johns Hopkins University. The work on marine forms, however, was carried on at the Harpswell Laboratory, South Harpswell, Me., and at Ocho Rios, Jamaica, B.W.I.²

1. PROTOPLASMIC MEMBRANES.

No topic in biophysics probably has been the subject of so much controversy as that of the plasma-membrane and its bearing on the phenomenon of permeability. Opinions upon it differ so widely that while its very existence is questioned by some workers, others positively assert that an actual morphologically and physiologically definite surface layer can be clearly demonstrated. Thus de Vries ardently supports its existence as a morphological entity, while Kite (23, p. 298) views it as a 'hypothetical' structure, and Fischer (15, p. 225) calls it a 'figment of the imagination'.

There are three main lines of attack on this problem, no one of which can by itself give conclusive evidence, although a combination of the

² Other difficulties in nomenclature, especially in reference to such common colloid chemical expressions as 'gel', 'conglutium', &c., are considered in the paper dealing with other physical properties of protoplasm already referred to (38).

³ I am indebted to Director J. S. Kingsley for the use of a room at the Harpswell Laboratory. To Frank Cundall, Esq., I am greatly obliged for his kindness in placing the facilities of the Institute of Jamaica at my disposal.

three does, it seems to me, give a very convincing if not irrefutable answer to the question. There is, first, the purely theoretical suggestion that some sort of membrane or septum is a physical necessity; that a membrane exists wherever two immiscible liquids come in contact. The retort to this contention is, that this is a question of definition, and that a surface tension membrane has nothing in common with osmotic membranes. A second method of attack on the problem is through the study of permeability. Most workers have concluded that a 'semi-permeable' membrane about a protoplast is a necessary prerequisite to differential permeability. The third mode of approach is through morphological data based on anatomical evidence of the presence of a definite structural membrane. It is with this third method that I shall deal.

If our discussion of the subject is to be at all intelligible it is necessary that we have a common understanding of the precise connotation of the word membrane. To the physicist a membrane is a solid structure, flexible in two directions and theoretically without thickness. A film, on the contrary, is a liquid structure, although even from physicists (34) do we hear of liquid membranes. Biological definitions of membranes run the whole gamut of physical possibilities, from a film two molecules thick to a tough parchment. Pfeffer, who is usually referred to as the chief advocate of the presence of a protoplasmic membrane, and who 'has shown remarkable ingenuity in the development of the plasma-membrane theory' (23, p. 282), was not certain of the exact nature of the membrane. In fact, in justice to Pfeffer, it should be pointed out that he adopted the term plasma-membrane 'for the questionable surface layer of protoplasm' (30, p. 189) in order to have 'a precise designation for the diosmotic layer', but regarded the 'expression plasma-membrane as a makeshift' which he 'would gladly drop if a knowledge of the structure and quality of the hyaloplasm would permit it' (29, p. 124). To Pfeffer, then, plasma-membrane was a convenient term for use in the consideration of permeability phenomena. It must be said, however, that Pfeffer in all probability believed in some sort of a definite protoplasmic membrane.¹

Von Mohl (26, p. 93) was one of the first to suggest that the 'primordial utricle' was, perhaps, limited without by a special membrane lying next to the cell-wall. (It is interesting to note that von Mohl cautioned against regarding the plasma-membrane as a solid layer sharply differentiated from the inner protoplasm.) De Vries recognized not only an external membrane, to which he gave the name 'ectoplast', but also an inner protoplasmic membrane, which he termed 'tonoplast', surrounding each vacuole. Recent workers such as Vonwiller (44, p. 288) and Prenant (32, p. 94) recognize the presence of a membrane around all cell inclusions, such as the nucleus, the

¹ 'In every case the limiting membranes determine whether or not a given substance shall be absorbed' (Pfeffer, 31, p. 92).

vacuoles, and 'the bodies of every kind contained in the protoplasm'. The ultra-modern tendency is—and I quote from Berczeller's (5, p. 61) work on the physical chemistry of membranes—'not to speak of "walls", "skins", or "membranes"'. We must not picture to ourselves a rigid layer, as the osmotic theory really demands but which has in animal cells never been observed; but we are dealing rather with liquid surface layers which in many cases . . . can also assume a solid form.' This reaction to the old notion of a plasma-membrane culminates in the school led by Fischer (15, p. 158), who believes that 'There are no membranes about cells'. Indeed, so fearful have some become of giving credence to the existence of a true protoplasmic membrane that they are now quite satisfied with a layer of but two molecules in thickness to which to ascribe all permeability phenomena.¹

Before discussing the experimental data which have led me to agree essentially with Berczeller in looking upon the plasma-membrane not as a skin, but as a highly viscous layer of modified protoplasm which may at times become quite fluid, it will be well to consider some membranes and surface layers which are pretty well recognized, chiefly with the view of excluding them from the discussion.²

Certain unicellular organisms are known to possess definite and readily isolated membranes; e.g. *Vorticella* has a tough membrane and *Euplotes* possesses a quite resistant pellicle. There is no contention over the existence of these structures. They may be said to be true membranes in the unqualified biological sense. There are, however, other unicellular organisms, such as *Paramecium bursaria* and many species of *Amoeba*, which are generally considered 'naked'. (Some *Amoebae* are said to possess a membrane of appreciable thickness. Vonwiller, 44, p. 286.) In marine ova there may or may not be an easily recognizable membrane. In *Fucus*, e.g., there is an egg-wall measuring 1μ in thickness. In *Asterias* and *Cerebratulus* the eggs are surrounded by a membrane which, on fertilization (*Asterias*) or escape into sea-water (*Cerebratulus*), lifts off to form the fertilization membrane. In the *Arbacia* egg there is said to be no discernible membrane, therefore the fertilization membrane must be a precipitation product or a new secretion (19, p. 239). These generally recognized and readily discernible membranes—'non-cellular secretions', which, in the zoological sense, are probably synonymous with the cell-wall of plants—are not the subject of the following discussion. We are concerned only with so-called 'naked' protoplasm, of which myxomycetes, most *Amoebae*, and all escaped protoplasm are examples. The same type of membrane which

¹ Personally, I cannot believe that a two-molecule layer, i.e. a surface tension membrane, is sufficient for any differential permeability phenomena.

² I prefer to keep the expression 'plasma-membrane' for the superficial layer of protoplasm. Little clarity will be gained by the substitution of any other name, old or new, until we have more real knowledge of the actual structure and composition of the plasma-membrane.

delimits such 'naked' masses of protoplasm is also to be found as a covering of the cell protoplast lining the cellulose walls of plant cells.

Having eliminated the true and readily visible membranes (fertilization membranes, pellicles of certain Protozoa, &c.) as not closely comparable with the plasma-membrane, we may proceed further to discard one or two suggested conceptions of the protoplasmic membrane. Bütschli's alveolar layer has been referred to as a possible plasmatic membrane. I do not regard this as a correct interpretation. The alveolar layer is merely a palisade arrangement of the superficial alveoli. This arrangement can hardly give to the layer any decided special properties not possessed by the alveolar protoplasm within.

It has also been suggested that possibly the hyaloplasmic border of myxomycetes, and likewise the ectosarc of *Amoeba*, is the plasma-membrane, or, at least, functions as the differentially permeable layer. Pfeffer (29, p. 123) has advanced this possibility. This may hold true in permeability phenomena, where not only the hyaloplasmic layer but the entire living colloidal system may perhaps function osmotically, but, as I have reiterated, it is not with permeability that I am concerned. Microdissection evidence indicates the presence of a delicate layer of protoplasm external to and more or less distinct from the peripheral hyaloplasm.

Chambers apparently inclines towards the suggestion of Pfeffer that the ectoplasmic layer is the protoplasmic membrane, and does not definitely acknowledge the occurrence of a plasma-membrane such as I have just described, i.e. a delicate layer more or less distinct from the ectoplasmic border. Chambers's expressions for what might be construed as a plasma-membrane are 'surface film' and 'surface layer', and the latter he makes synonymous with ectoplasm (8, p. 4; 9, p. 11). Only when the ectoplasm becomes an exceedingly thin layer, as in 'naked' marine ova, is it then even loosely comparable to a plasma-membrane. Chambers's 'surface layer' is not, even when thin, strictly a film or membrane (although he (11, p. 46) occasionally uses the word membrane), for he recognizes no line of demarcation between the surface layer and the inner plasm. The surface layer, as viewed by Chambers, is a region which 'merges insensibly' into the cell interior (11, p. 45). Pfeffer expresses the possibility of this when he says that the more dense layer of the hyaloplasm is 'probably only an outer zone' (29, p. 123), and 'A definite delimitation of the plasma-membrane from the inner layers of hyaloplasm is not probable', unless a more thorough knowledge of the structure of the hyaloplasmic border should permit such an interpretation (29, p. 124).

With this knowledge of some of the many divergent conceptions of the plasma-membrane we are better prepared to interpret the following data:

The Living Membrane. If the hyaloplasmic border of a plasmodium which is in the active stage—that is, if it is or has recently been streaming—

and therefore is of low viscosity, is pierced by a microdissection needle and the needle is slowly moved toward the edge of the protoplasmic mass, the liquid hyaloplasm will follow the needle until a good-sized artificial pseudopodium is produced. The formation of so large a pseudopodium necessitates considerable increase in surface of the plasmodium in that region, to accomplish which the outer layer must either be in a liquid state or, if solid (i. e. a gel), must be capable of being greatly stretched. That the membrane is not elastic is readily determined when such a pseudopodium is being formed by releasing the needle and observing the absence of any appreciable contraction. The outer layer, therefore, must during pseudopodium formation, be in a liquid state. Increase in surface is accomplished by additions to the outer layer from the liquid hyaloplasm. There is no stretching in the sense of an elastic membrane, no great separation of the surface particles, but, as in any liquid film, with increase in area more particles are forced into the surface layer.

The experiment so far has proved only that the plasmodial surface is at pseudopodium formation liquid; but that it differs from any other liquid surface has not been shown. Optically the liquid surface layer of a plasmodium is much more refractive in comparison to the interior protoplasm than is, for example, the liquid surface of water in comparison to its interior. In observing these liquid surfaces of streaming protoplasm one constantly gets the optical impression that the outer layer differs, and is sharply delimited from the bordering hyaloplasm.

The advance of such an artificially produced pseudopodium will at some point suddenly be halted by a change in consistency of the protoplasm, and *this pronounced and sudden change takes place at the surface*, for the inner hyaloplasm increases but little in viscosity. Subsequent advancement of the needle causes a break in the surface. The break is not sudden, however, for there is now some actual stretching of the firm (gelated) surface layer, which ultimately tears apart.

From these observations we may justly conclude that the surface layer of an advancing myxomycete pseudopodium is liquid, while that of an inactive one is firm. When a quiescent, and therefore more viscous, plasmodium is dissected in the manner above described there is no flow of protoplasm. The surface layer is, after some stretching, ruptured. We have here then a reversible solation-gelation phenomenon. The inactive surface layer is a highly viscous emulsion colloid, undoubtedly in the gel state,¹ which solates (i. e. becomes a sol) when streaming takes place, and reverts to the gel state when the plasmodium again becomes inactive. This

¹ The only criterion here for a gel or sol state is viscosity. This does not furnish conclusive evidence, but where the viscosity is so very high or so very low one can be reasonably certain that the protoplasm is, in colloidal structure, actually a gel in the first case, or a sol in the second case. For a more complete discussion of this see 32.)

surface layer is exceedingly delicate, of immensurable thickness, and is not identical with the hyaloplasmic border (or ectoplasm), which, to be sure, it resembles in constitution, but from which it is more or less sharply delimited.¹

The following extract from notes on my experiments shows how closely the behaviour of the surface layer of *Amoeba* resembles that of myxomycete plasmodia, and, like the latter, supports the theory of a delicate protoplasmic membrane.

‘That portion of the membrane of new pseudopodia of *Amoeba* which is immediately concerned in the advancement of the pseudopodia—that is, the region of the membrane at the foremost tip of an advancing pseudopodium—is in a state of fluidity, while those regions of the membrane bordering the more or less quiet portions of the *Amoeba* are of very high consistency, undoubtedly a colloidal gel. The rapidity with which the liquid membrane of an advancing pseudopodium will gel is beautifully seen in the result of a rapid stretching of it. If the membrane of a quiescent *Amoeba* is torn it is found to have the consistency of a gel, and, though elastic, it tolerates only a moderate amount of stretching. On the other hand, the liquid membrane bounding the advancing part of a moving *Amoeba* behaves at first, if the dissection be quickly performed, exactly like the liquid membrane of a water droplet, but extension of the liquid membrane is possible for only a brief space of time, for gelation quickly takes place, after which the now gelled membrane may be stretched a bit, when it breaks.

The behaviour of the ectoplast is quite similar to that of the membrane, in this respect differing markedly from the hyaloplasmic border of myxomycetes. The ectoplasm of *Amoeba* is in a liquid state when actively flowing, but when quiet is of high consistency, though it never attains the rigidity of the passive membrane. It is this difference in density and consequent difference in degree of extensibility which makes it possible often to distinguish the membrane from the ectosarc even in the living condition.

The resistant, elastic, and highly viscous gel nature of the surface layer of *Amoeba* is evident from the following experiment. An active specimen of *Amoeba* was twice partially severed, leaving but a strand (apparently double) of protoplasm, the plasma-membrane, connecting the two halves. Subsequently, after the needles were removed, the two halves were drawn towards one another by contraction of the connecting elastic gel membrane, and on coming in contact they re-joined.’

¹ De Bary's notion of the plasmodial membrane was also essentially this. He says (3, p. 42): ‘These facts . . . do not permit it (the plasma-membrane) to be considered as a skin differentiated from the ground-substance, but it is to be looked upon as a special superficial layer of the living plasmodium, from which it is usually readily distinguishable.’

These observations support the theory of amoeboid movement advocated by Hyman, who says (21, p. 88): 'Since the ectoplasm is a more or less rigid gel, the direct cause of pseudopod formation must be a local liquefaction, and the direct cause of the withdrawal and contraction of pseudopodia must be coagulation. This gelation and solation are the essential processes in amoeboid movement.' I believe, however, that it is probably primarily the membrane which functions in this solation-gelation phenomenon, since it becomes much firmer on gelling than does the ectoplasm.

A further bit of experimental evidence tending to show that the surface of protoplasm is far more viscous, resistant, and elastic than the interior, is to be had by tearing apart a mass of inactive and very viscous protoplasm. If two needles are placed within the highly viscous protoplasm of a myxomycete plasmodium and are then separated, the protoplasm will, as the limit of extensibility is approached, tear very much as bread-dough does when pulled apart, but there is almost invariably a remaining strand which persists for some time after the mass as a whole has separated. This remaining strand may be stretched to a very fine thread, exhibiting a surprisingly high extensibility. This persistent thread of protoplasm is *always* from the surface of the torn mass. If, now, the one needle is returned and again put into the protoplasmic mass near the ragged surface from which the membrane just described has been torn (the highly viscous and partly degenerate condition of the protoplasm prevents the formation of another membrane, i. e. the wound is not healed) and the two needles are again separated, the mass of protoplasm, when its limit of extensibility is reached, tears apart abruptly and cleanly. There is now no persistent outer layer of more viscous, resistant, and elastic protoplasm.

One of the fundamental properties of the living substance is the capacity instantly to surround itself with a membrane such as that which has just been described. Tears in the plasma-membrane are usually instantly repaired. When the surface of a plasmodium or of an *Amoeba* or an ovum is torn, it is, if the protoplasm is normal, immediately healed, and there is seldom any escape of protoplasm. This capacity exists even in the ova of *Fucus*, where the outer layer is not a membrane but a pliable wall of considerable thickness. A tear in this wall is rapidly repaired, the new covering being to all appearance identical with the old.

This capacity for forming protective membranes is to be observed in the behaviour of escaped masses of protoplasm. For example, ejected masses of protoplasm from pollen tubes sometimes develop membranes immediately on being freed. (The behaviour of escaped protoplasm from growing pollen tubes varies greatly. It is sometimes immiscible in the surrounding medium of water and immediately forms a membrane on being freed, while it equally often diffuses rapidly into the water with no indication of the formation of a membrane.) The membrane formed is surprisingly

tough. Fragments of it can be dragged into the plasma mass. (This is, of course, the gelated, degenerate membrane. The *living* membrane *cannot* be separated from the protoplasmic mass.)

The highly viscous, inactive protoplasm from the hyphae of the bread-mould *Rhizopus*, when exposed by a rupture or when forcibly ejected through a tear in the filament of the mould, forms no membrane. Its high gel consistency is apparently not conducive to membrane formation. That protoplasm of gel consistency is incapable of forming a membrane is not surprising, since a not too high viscosity is a physical prerequisite to a readjustment of particles. On the other hand, very dilute protoplasm does not always form droplets, with apparent membranes, when freed. This truth is well illustrated in the behaviour of the protoplasm of *Amoeba*. The ectoplasm of *Amoeba*, though very viscous, will invariably, if normal, round up into small spheres when bits of it are isolated, while the endoplasm which is of low viscosity does not round up when freed nor form a protective surface, but mixes with the surrounding medium.

This behaviour suggests the view of de Vries that the plasma-membrane is formed only from the ectoplasm. De Vries looked upon the hyaloplasmic border of myxomycetes and *Amoeba* as the 'organ of cell membrane formation'. That the plasma-membrane is made from hyaloplasm (matrix) and not granular protoplasm is evident from the fact that it is hyaline and possesses no granules. That it is usually formed from the hyaloplasmic border of myxomycetes and the ectosarc of *Amoeba* is likewise very evident in view of the fact that these regions are external to the internal granular plasma and therefore are the first to come in contact with the surrounding medium when the surface is torn. But that the ectoplasm is the 'organ' of membrane formation is not without experimental disproof.

It is generally true that a rupture in the surface of *Amoeba* which leaves ectoplasm exposed is quickly covered over without much if any loss of protoplasm, and, further, small globules of ectoplasm pinched off readily round up and maintain their identity, although their viscosity is considerably higher than that of the endoplasm. The endoplasm, on the other hand, when exposed to the surrounding medium by a deep wound, usually flows out and mixes in the medium, and apparently never forms a protective membrane unless a surface layer of ectoplasm is first established. I have, however, observed so rapid a transformation of the freshly exposed surface of liquid endoplasm that granules are caught in the membranous gel. What usually takes place when an *Amoeba* is torn by a deep rupture and the liquid endoplasm is not lost by dispersion is, apparently, a rapid conversion of the peripheral granular plasma into a more viscous granule-free layer, the ectosarc, which, in turn, at its surface is converted into the highly viscous

membrane. A deep tear in a myxomycete plasmodium will often result in the formation of globules of a hyaline substance with characteristic membranes, and these clear masses of substance are formed not from the peripheral hyaloplasm but from granule-free protoplasm which comes from the very centre of the plasmodium. Thus, it is apparently the hyaline matrix, whether peripheral in location or not, which is capable of forming a membrane in myxomycetes. (Whether or not the peripheral hyaloplasm of myxomycetes is identical with the matrix of the granular plasma, differing only in location, cannot be said, but the two are not as highly differentiated as are the ectoplasm and endoplasm of *Amoeba*. In the ciliate *Euplotes* the differentiation between the two regions is still greater.)

The capacity for membrane formation persists only as long as the protoplasm is normal (although this property of membrane formation is one of the last to be lost in dying protoplasm). This fact is well illustrated in the behaviour of escaping protoplasm, from a bread-mould hypha or a filament of *Vaucheria*, for example. The first protoplasm which streams from a rupture almost invariably forms protective membranes (unless the escaping protoplasm be of very high viscosity, as is true of the quiescent protoplasm in *Rhizopus*). These liquid membranes at first readily increase in area as the droplet of escaping protoplasm increases in volume, but later they suddenly gelate and rupture from pressure of the inflowing protoplasm. The physiological change which has caused the gelation of the membrane surrounding the freed protoplasm had also robbed the now degenerate escaping protoplasm of the capacity to form a membrane.

The evidence so far presented favours the belief in a protoplasmic surface layer which is usually in the gel state (i.e. firm in consistency). This is also Chambers's contention (11, p. 45). But to Chambers the surface layer of so-called 'naked' protoplasm is frequently of 'an appreciable depth'—that is, the degree of viscosity (of the protoplasmic surface layer) is greatest on the outer surface and least on the inner surface. Thus does the high consistency at the surface grade insensibly into the very fluid condition of the interior. When the surface layer is sub-microscopic, such as may form over protoplasm which has freshly come in contact with water, then Chambers's surface film is identical with the protoplasmic membrane as I have described it. But when Chambers regards the ectosarc of *Amoeba*, which may attain a thickness of 10 or more micra. as the plasma-membrane, I cannot agree with him. On the contrary, I believe that the hyaloplasmic border of myxomycetes, the ectosarc of *Amoeba*, and the surface of all so-called 'naked' protoplasm is possessed of an ordinarily highly viscous outer protoplasmic layer of such delicacy as to be immensurable, but which does not necessarily grade imperceptibly into the more liquid condition of the interior. This outer layer is sometimes so definitely delimited from the inner protoplasm as to be optically distinguishable as a definite membrane.

Though this is with difficulty seen in the living condition, it is often readily to be seen in degenerate protoplasm.¹

The Degenerate Membrane. The observations so far presented have had to do almost entirely with the living plasma-membrane. Although the evidence for the presence of an outer more viscous protoplasmic layer seems to me to be conclusive, it must be admitted that this layer cannot be irrefutably demonstrated to be, in the living state, a definite membrane quite distinct from the protoplasmic mass which it bounds. The structure is such a delicate one, and is, after all, so little different from the inner protoplasm itself (of which it is when liquid so intimate a part), that separation of the living membrane by dissection is a physical impossibility.

The reactionary attitude of our science to observations on dead material is a healthy one. The criticisms directed against any evidence which purports to prove the existence of a living membrane by the presence of a dead one are legitimate. For example, the dead structure may be an entirely new product—perhaps a precipitation is formed as a result of death. That this is a possibility, I grant. But the dead membrane is to all appearances so evidently a structure having the same position and dimensions as that which can sometimes be observed on living protoplasm, that it is worthy of some consideration as evidence of the existence of a membrane about the living substance.

If the escaped protoplasm from a pollen tube, of *Iris* e.g., is allowed to gel—that is, to degenerate—the membrane formed at the time of ejection becomes fixed (possibly coagulates). It can then sometimes be isolated from the protoplasmic mass and dragged about as one would handle a film of scum such as forms on boiled milk.

In a myxomycete plasmodium one frequently finds large globular masses that are quite distinct from the larger protoplasmic mass in which they rest. The protoplasm which they contain is, so far as can be determined, identical with that of the plasmodium proper. These globules can be isolated and torn open, and when partly emptied they are found to be sacs bounded by a delicate membrane which on dissection is found to be a tough rigid gel. Before isolation, when the globule is handled while still within the plasmodium, the membrane is soft and exceedingly sensitive, often breaking before a perceptible indentation can be made. The change from this state to that of the isolated membrane is the result of degeneration and gelation.

¹ Sharp delimitations between protoplasmic regions are common enough in organisms. That such structures as the nucleus, chromatophores, &c., are sharply delimited from the surrounding protoplasm is evident, but even less specialized regions such as the ectoplasm may be sharply delimited from the rest of the protoplasm. Of the ectoplasm of *Eupletes*, Taylor (12) says: 'Frequently, there is evident a fairly definite boundary between the ectoplasm and endoplasm, but this condition apparently varies. If (upon disintegration) its outflow be not too rapid, the endoplasm separates from the ectoplasm . . .'

The membranes so far discussed have been those on 'naked' masses of protoplasm, such as myxomycetes and *Amoebae* and escaped protoplasm. It has long been believed that protoplasm within a cellulose wall is bounded by a membrane which by Pfeffer and others has been looked upon as the osmotically functioning region of the plant cell.

This outer protoplasmic layer next to the cellulose wall de Vries termed the ectoplast. Direct evidence of its presence in the living condition is very difficult to obtain. I have, however, observed it after degeneration and consequent coagulation have made it coherent and rigid enough to be separated from the protoplasm.

The hyphae of the bread-mould *Rhizopus* are frequently very turgid. Occasionally a filament can be found in which the protoplasm is a firm jelly and is sufficiently turgid to cause a slight protrusion of the protoplasm from the hypha when this is ruptured. By pressure with a needle some distance behind the torn end, the rod of protoplasm can be made to ooze out like oil paint from an artist's tube. This protoplasmic jelly is sufficiently rigid to hold its shape until dissected. It remains immiscible in the watery medium. Dissection will sometimes reveal, next to the protoplasmic rod, a delicate membrane which on further dissection proves to be a thin outer layer of highly viscous, gelled protoplasm enveloping the rod, and far more rigid and resistant than the soft protoplasmic jelly within. This thin membrane may be partially freed from the rod of protoplasm. I know of no other satisfactory designation of this firm outer layer than that it is the ectoplast, the plasma-membrane (in a degenerate, gelled state).

The Vacuolar Membrane. I have referred to the difference in behaviour of freed protoplasm from pollen tubes. Frequently, the protoplasm mixes with the surrounding water immediately on escaping. If the larger vacuoles thus set free by the escaping protoplasm of a germinating pollen grain are observed, they will be seen to be surrounded by a thin but plainly visible protoplasmic membrane. Clinging granules can be seen to glide along its surface. This membrane permits the vacuole to maintain its identity for several seconds while free in water. The volume of the vacuole slowly increases, due to osmosis, until it becomes twice its original size, when it bursts.

The vacuolar membrane may, when exposed to water, undergo a decided change. The many small vacuoles ejected with the liquid protoplasm from a hypha of the bread-mould *Rhizopus* are found, when exposed to water, to possess a quite resistant membrane which tolerates considerable pressure. But when these vacuoles are handled while still within the mass of protoplasm they burst at the slightest touch.

This view of the vacuolar membrane as a somewhat differentiated layer of protoplasm closely comparable with the external plasma-membrane

rather precludes looking upon the vacuole as an autonomous or permanent organ.¹

The behaviour of the contractile vacuole of certain Protozoa, as apparently first described by Wrzeńskiowski (45 and 46), to which my attention was called by Dr. C. V. Taylor, who has observed the same temporary existence of the contractile vacuoles in *Euplotes* and *Paramoecium*, quite does away with the possibility of looking upon the contractile vacuole of Protozoa as a permanent organ, and therefore precludes the existence of any sort of permanent protoplasmic membrane surrounding the vacuole. It seems (45, p. 162)² that the contractile vacuole in certain Protozoa is formed by the fusion of several smaller vacuoles, and on ejection of the vacuolar contents to the exterior, a *new* vacuole is formed by the fusion also of several smaller ones, and the formation of the new vacuole may be—in fact, usually is—initiated before the complete disappearance of the old discharging vacuole.

The fusion of the vacuoles and the temporary life of the ultimate contractile vacuole make it difficult to conceive of the vacuole as so intricate an apparatus as Stempel (39, p. 458) describes it. But especially does the fusion of the vacuoles indicate the temporary and facile character of the 'wall'. If the vacuolar membrane is at any time a rigid gel, solation apparently takes place on the fusion of two vacuoles and also on contraction of the ultimate vacuole.

Thus is the vacuolar membrane, like the peripheral cytoplasmic membrane, essentially protoplasm, fluid in consistency, and if at times it is in the gel state then it is readily reversible.

The Nuclear Membrane. The most beautiful demonstration of a protoplasmic membrane (using protoplasm in its broadest sense) is to be had by dissecting the isolated nucleus of an *Amoeba*. The nuclear membrane is generally believed to be a more definite and more readily distinguishable structure than the surface membrane of cytoplasm. This may be true, but the nuclear membrane cannot in the living state be any more readily distinguished or isolated than can the outer cytoplasmic membrane.

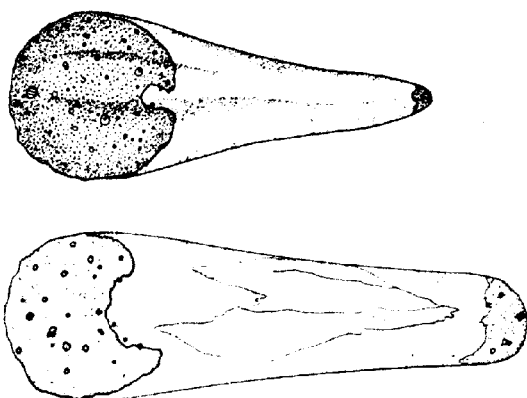
Kite (22, p. 6) describes the nuclear membrane of the eggs of *Asterias*, *Cuminga*, &c., in these words: 'This structure is a concentrated tough gel of relatively high viscosity and is not to be confused with the hypothetical surface of vacuolar plasmatic membranes.' Chambers (9, p. 10) supports Kite with the statement: 'Evidence that this membrane is a morphological structure is shown on withdrawing some of the nuclear contents with a micropipette.

¹ Arthur (1, p. 499), in describing 'the variety of catastrophes which overtake the moving vacuoles' in the hyphae of bread-mould, says, 'I cannot see . . . any ground of support for the supposed autonomy of the vacuoles and a special vacuolar membrane advocated by de Vries, Went, Wakker, Bokorny, and others.'

² This résumé was taken from the article in Russian by Wrzeńskiowski (45), but reference is also given (46) to the more extensive work in German.

The nucleus then partially collapses, throwing the nuclear surface into irregular folds.' This is, of course, only evidence of the fact that the nuclear membrane, like the plasma-membrane, may at times exist in the gel state, although here I doubt very much if a nucleus subjected to such treatment would long remain normal. The folded nuclear surface may be coagulated.

Whether or not we are justified in calling the nuclear membrane a morphological structure, any more than that expression is applicable to the cytoplasmic membrane, is doubtful. Chambers (9, p. 10) describes an experiment in which he cut the nucleus of an ovum in two. Each part



The isolated, degenerate *Amoeba* nucleus from which the coagulated nuclear membrane has been partially separated.

rounded up into a droplet. On coming in contact the droplets ran together. This observation restricts our notion of a morphological structure as applied to fluid membranes.¹

The accompanying figure illustrates how nicely the nuclear membrane can be torn off after isolation, and hence after degeneration of the *Amoeba* nucleus. Staining with methyl blue intensifies the delicate membrane. The nuclear substance after isolation degenerates into a coagulum into which the microdissection needles are placed. As the needles are separated the nuclear membrane tears off as a thin though quite resistant veil.

Physical Chemical Evidence bearing on the Existence of a Plasma-

¹ Botanical cytologists, working on fixed and stained material, have viewed the nuclear membrane as a very definite and readily distinguishable structure when dead. Mottier (27, p. 191) describes how the nuclear membrane of pollen mother-cells of *Lilium* appears to be made from the brilliant kinoplasm, and Yamanouchi (47, p. 431) describes how the membrane of the original nucleus in the tetraspore mother-cell (of the red seaweed *Polyphenta*) persists through the two mitoses.

membrane. The colloidal nature of the plasma-membrane, its structure and composition, how it is formed, and how it functions are all problems of great interest, but they are chiefly problems of colloidal and molecular physics, and therefore primarily of so theoretical a nature that few irrefutable statements can be made regarding them. However, the physical chemical evidence bearing on the existence of a plasma-membrane is of so fundamental, even though theoretical, a nature that it deserves very careful consideration.

From familiar surface tension phenomena we know that the surface of a liquid is the seat of certain physical forces which give to the surface layer a structure differing from that of the interior. Furthermore, all chemical reactions are surface ones; consequently, we can conclude that the region of contact between two immiscible systems is likely to be one of both physical and chemical activity. The chemical and physical forces active in the septum separating two fluid systems is often sufficient to convert the septum into a third system differing physically and chemically from either of the two systems which it separates. It would be very extraordinary indeed if so intricate a colloidal system as protoplasm should prove an exception to this and be quite inert at its surface. Quite the contrary condition is the likely one. The extremely complex nature of protoplasm suggests the occurrence of an unusual surface activity. We are led to expect this through consideration of those peculiar instances where substances, like colloidal (spongy) platinum, are active in the colloidal state, though they are inactive when not in this state.

The theorem of Willard Gibbs tells us that any dissolved substance which lowers the surface tension of a solution is deposited at the surface, i. e. is positively adsorbed. Conversely, the concentration of any substance which raises the surface tension is less at the surface. Such selective adsorption results in the production of a region which, without any actual chemical reaction, differs in chemical make-up from either of the two original systems. But adsorption is always a preliminary to chemical action (although chemical union need not always follow adsorption. Whether adsorption is purely a physical union or a chemical one is simply a question of degree of firmness of the bond. A loose chemical union and a firm physical one are in this respect the same). Consequently, there occur in the region of separation chemical activities peculiar to the septum alone, which, in turn, produce substances foreign to either of the systems which the septum separates. Just how far this process goes in the formation of the plasma-membrane cannot be said. But, realizing the possibilities which so complex a colloidal system as protoplasm offers to physical and chemical surface activity, there is every justification for assuming, *a priori*, that the septum which separates protoplasm from its surrounding medium is itself a system differing in chemical composition as well as in physical structure from both of the systems which it separates.

I have spoken only of surface forces. That these are sufficient to satisfy all membrane activity is unlikely. The state of equilibrium which is established in the membrane, and to perhaps a less degree in the hyaloplasmic border, is upset by extension and contraction. What forces are concerned in re-establishing this equilibrium we can only conjecture. But in considering them one must not lose sight of the fact that the organism is full of regulatory reactions, and an explanation of membrane formation based simply on surface phenomena is undoubtedly inadequate.

Comparison of the Plasma-membrane with Precipitation Membranes.

Just how closely the plasma-membrane is to be compared with those precipitation membranes which are formed when two substances such as CaCl_2 and K_2CO_3 , or CuSO_4 and $\text{K}_4\text{Fe}(\text{CN})_6$ are brought together is a matter open to discussion. These precipitation membranes and the plasma-membrane have much in common, but also differ in some very fundamental properties. (I am adverse to making any distinction between the living and the non-living which tends to ascribe to the living any properties not possessed by the non-living, other than such as result from the great complexity of the former. This complexity, or organization as it is sometimes called, is, however, a distinguishing characteristic of the living, and we have as yet no satisfactory explanation of it. The living membrane manifests this organization—that is, it exhibits certain self-regulatory reactions not possessed by the non-living membrane.)

Comparison of the plasma-membrane and the classical Traube precipitation membrane of $\text{Cu}_2\text{Fe}(\text{CN})_6$ is misleading in at least one respect. The former is a reversible gel,¹ the latter an irreversible one; the former grows by reversion to a sol and the addition to the surface of colloidal particles from the interior; the latter (the Traube membrane) grows by rupture of the surface and the formation of a new membrane. Hober (20, p. 64) says, 'The plasma-membrane must probably be a thin surface layer comparable to a Traube precipitation membrane'. This may be true in a very broad and general way, but Hober's further statement, that as the Traube membrane grows so does the plasma-membrane, is not true if the comparison is to the method of growth by rupture and subsequent healing which is characteristic of the typical Traube membrane. Growth of the plasma-membrane is accomplished by a deposition of colloidal particles (and possibly substances in molecular dispersion) at the surface between the particles already at the surface which become somewhat separated as the area of the fluid membrane increases. (The deficit of colloidal particles just below the surface, which results from this surface deposition, is replenished from within by diffusion.) A better analogy is to be had in the remarkable precipitation membrane produced by bringing HCl and

¹ Bechhold's statement (4, p. 56), 'We may describe membranes as *irreversible gels*', if in reference to living plasma-membranes, is quite untenable.

Na_2SiO_3 together (35, p. 681). This membrane may remain liquid for months. The duration of liquidity of most of these precipitation membranes ranges from 3 to 120 seconds. Quincke (36, p. 722) describes these membranes as 'a very thin invisible film of an oily liquid precipitate'. In these physical, as well as in some of their osmotic properties, these precipitation membranes are very similar to the plasma-membrane. Whether or not the protoplasmic membrane is a precipitation membrane, one cannot say definitely. I am inclined to believe that it is not, primarily because of its ready reversibility.

Thickness of the Plasma-membrane. As yet no mention has been made of the thickness of the plasma-membrane except to say that it is very delicate. Any attempt to estimate its thickness (after it is isolated and has coagulated) with the aid of a micrometer, the very graduating lines of which exceed in breadth the apparent thickness of the membrane, will, of course, be very crude. Yet I made such attempts and estimated the thickness to be less than one-fifth of a micron. This value is not far from that given by Quincke for the thickness of some of the precipitation membranes with which he worked. He says (34, p. 630), 'The thickness of this oil layer can, according to my investigation, be so small, less than 0.031μ , that it can no longer be perceived with our best microscopes'.¹

Dewar (13, p. 16), working on soap films, gives the far more minute thickness of soap bubbles 'thinned to the "black" stage' (this closely approaches the minimum thickness of such films) as ' 1.5μ 's.' Bechhold (4, p. 34), in reporting the work of others, also gives a very low figure. To quote: 'The thickness of the layer which will just form a solid skin has been measured, and found to be, for peptone 3μ (Metcalf), for albumin 3 to 7μ (Devaux). Thus it is probably many times greater than the hypothetical diameter of a molecule, perhaps even equalling the radius of molecular attraction.' That the value may not only equal but exceed the radius of molecular attraction is suggested by Quincke (35, p. 631), who states that.

¹ I should like to call attention to some statements on the limit of size of objects visible and measurable with the modern microscope. Czapek (12, p. 25) states that 'Ordinary microscopical observation with the strongest lenses can show particles of about 250μ in diameter'. One quarter of a micron (250μ) is not only not the limit of visibility of the strongest lenses, but is actually within the limit of fairly accurate mensurability. Quincke (34, p. 630) remarks, as quoted above, that the thickness of a precipitation membrane is less than 0.1μ , which cannot be perceived by the best microscopes. I cannot agree with either Quincke or Czapek, although Quincke's statement is much nearer what I find to be true, namely, that the limit of visibility is about, but probably somewhat less than, 0.0001 mm . (0.1μ). The value given by Taylor (43, p. 42) is somewhat above this. He says, 'A sol whose particles are less than about 0.15μ will not be recognizable even with the best microscope' (magnification 2,250). An extremely low value is given by Burton (6, p. 117) in a table of 'Lower limits of diameters of small particles'. He gives the size of a particle visible under the 'ordinary microscope' as $2.5 \times 10^{-6} \text{ cm}$. (0.25μ is also given, but this is undoubtedly a typographical error, since 25.0μ and not 0.25μ equals $2.5 \times 10^{-6} \text{ cm}$). This value in micra is 0.025μ . Possibly Czapek had this figure in mind when he gave 250μ as the limit, but misplaced the decimal point. We may safely conclude, then, that the limit of microscopical visibility is not above 0.1μ .

'This border value (i.e. the thickness of the membrane upon which certain surface-tension values depend) is just twice the effective radius of molecular forces . . .'. These suggestive values are mere analogies, but there is good reason to suppose, because of the similarity in physical properties and method of formation, and of actual, albeit rather crude, estimation, that the thickness of the plasma-membrane is of the order of magnitude of precipitation membranes, namely 0.0001 mm. (0.1 μ).

It is of value as well as of interest to consider the findings of physicists who step temporarily into the field of biology to work upon living material in order to compare the phenomena observed there with similar phenomena seen in the inorganic world. Quincke (34, p. 629), in working upon the cells of *Chara*, *Elodea*, &c., found that the two masses of protoplasm which have become separated as a result of plasmolysis of a cell do not always unite on coming together when the protoplasm swells, but are separated by a film similar to that which surrounds the entire protoplast. Comparing the physical properties of this film with those of solid and liquid lamellae of inorganic precipitates, Quincke concludes that this protoplasmic film is 'a very thin liquid membrane'. Lehmann (24, p. 409) also believes that the comparison of the liquid membranes of artificial cells with the living plasma-membrane is of more than merely superficial significance.

Attempts on my part to establish the presence of a plasma-membrane through observation with the ultra-microscope were unsuccessful. Though it was possible to observe the vibration of colloidal particles in the hyaline matrix of myxomycetes, there was no distinct outer layer discernible by aid of dark ground illumination.¹

SUMMARY.

1. The direct evidence from microdissection indicates the existence of a plasma-membrane on the surface of all protoplasm.
2. The indirect evidence derived from a consideration of surface phenomena strongly supports the belief in a differentiated surface layer of protoplasm.
3. The plasma-membrane is essentially protoplasm, although it differs in physical properties, and probably also in chemical constitution, from the mass of plasma which it bounds.

¹ Gaidukov (16, p. 50, in connexion with his observations on botanical material by means of the ultra-microscope, says, 'The cell membrane exhibits a pronounced optical inhomogeneity, which does not interfere with the investigation of the cell contents'. The last clause of this sentence makes one wonder if Gaidukov actually has reference to the plasma-membrane. The German word 'Zellmembran' is synonymous with 'Zellhaut', which is the 'Plasmahaut' or plasma-membrane. It is unfortunate that he does not describe his observations in greater detail if he was actually able to distinguish the protoplasmic membrane by aid of dark-ground illumination. He (16, p. 74) does, however, say, 'The hydrosol complex of protoplasm (cytoplasm) is protected by a hydrogel layer (plasma-skin) . . .'.

4. The plasma-membrane is commonly of high viscosity, undoubtedly a gel, but it readily reverts to the liquid sol state.
5. The plasma-membrane is, above all, facile. It is capable of ready adjustment to changes in contour and area.
6. One of the characteristic properties of the living substance is the capacity to form, almost instantly, a membrane on its surface. The few apparent exceptions to this have to do with protoplasm of extreme liquidity.
7. The living membrane, though rather sharply delimited from the inner plasm, is not capable of isolation.
8. The degenerate, coagulated plasma-membrane *can* sometimes be isolated. It is then seen to be of very firm consistency, elastic, and exceedingly tough.
9. The nucleus and vacuoles also possess protoplasmic membranes which are closely similar to the outer plasma-membrane.
10. The thickness of the plasma-membrane is probably about 0.1μ .

II. THE NON-MISCIBILITY OF PROTOPLASM IN WATER.

If we accept a simple, non-technical definition of the term miscibility we may then proceed with satisfaction to a discussion of the problem of the miscibility of protoplasm in water. If, however, we try to base our definition on the molecular physics of the phenomenon we are likely to get into difficulty, because our problem will become so involved with the physics of diffusion and imbibition that the original simple question, Is protoplasm miscible in water? will be completely obscured in a theoretical discussion. In order, however, to arrive at a definition of miscibility which will avoid danger of confusing the latter with the closely related phenomenon of imbibition it will be necessary to discover to what extent one is justified in distinguishing between the two phenomena.

When a dry reversible gel, such as glue, is wetted, there is first a pronounced swelling, but the individuality of the original piece of glue is not lost. The piece has merely increased in volume. So far, only imbibition, i.e. absorption of water and consequent swelling, has taken place. But as absorption increases the original glue mass begins to disintegrate, and ultimately we have an aqueous dispersion (colloidal solution) of glue. Imbibition has given way to diffusion, and the merging of the one phenomenon into the other was gradual and imperceptible. In the last analysis the molecular physics may be the same in both cases, the distinction between the two phenomena being merely one of degree.

It is quite possible that the behaviour of protoplasm in relation to water is closely analogous to that of glue, and we are thus put to it to decide whether the taking up of water by protoplasm is to be regarded as

a process of diffusion or one of imbibition. Consequently, if we define miscibility as capability of diffusion, a solution of the problem of the miscibility of protoplasm in water resolves itself into the problem just stated, namely, Is the taking up of water by protoplasm a phenomenon of diffusion or one of imbibition? Should we decide that it is an imbibition process, are we then justified in looking upon protoplasm as water-miscible? The former question is not readily nor irrefutably answerable. As for the latter, I think that we are quite justified in saying that miscibility and imbibition are not, in certain important respects at least, identical. It would be carrying our interpretation to the point of absurdity to grant, e.g., that a blotter or a sponge is miscible in water.

The question, Is protoplasm miscible in water? is, then, best considered first in its simplest form, taking a dictionary definition of miscibility. If miscibility means capability of mixing, does protoplasm mix with water when the two are brought together?

To begin with, we know of certain so-called 'naked' masses of protoplasm, such as *Amoeba* and myxomycete plasmodia, which live in water. Self-preservation of the organism, therefore, would demand that this protoplasm be immiscible in water. But we are told that these masses possess an outer layer which protects the protoplasm. To this one can justly reply that the outer layer is itself protoplasm, differing somewhat from the inner protoplasm, to be sure, but essentially protoplasm. (If, of course, the plasma-membrane is a typical precipitation membrane, and therefore an entirely new product, which, however, I do not believe to be the case, then the above reply is not valid. Chambers, who supports the view that protoplasm is miscible in water, must, since he makes surface layer synonymous with ectoplasm (8, p. 4), admit that this surface is essentially protoplasm. What Fischer holds regarding the nature of the outer surface of protoplasm I cannot say. He (15, p. 1,38) does not, however, look upon it as a membrane. Without further qualification this hypothesis of the absence of membranes about cells precludes the miscibility of protoplasm.) But if there is some objection to looking upon this outer layer as protoplasm, or if it is said that its immiscibility is not proof of the immiscibility of the inner protoplasm, we can carry our investigation farther and tear open some cells and see what happens when a fresh surface of protoplasm is exposed to water. This simple and direct method of deciding such a problem was the one employed by the pioneer workers on protoplasm, who were not greatly influenced by theories in molecular physics and colloid chemistry. The keenly analytical observations of early investigators, because of the simple and direct methods employed, are, it seems to me, of more than historical interest.

The first mention of 'sarcode', the original zoological name for protoplasm, is in Dujardin's '*Recherches sur les Organismes inférieurs*' (1835).

He there (14, p. 367) describes the 'living jelly' as a 'transparent, glutinous substance, *insoluble in water*' ('cette substance glutineuse, diaphane, insoluble dans l'eau'), and further (14, p. 368) emphasizes 'its insolubility in water' as one of the properties which distinguish it from 'substances with which it might be confounded', such as 'albumin, which it resembles in its mode of coagulation'. But one does not have to go back nearly a century for authoritative evidence on the immiscibility of protoplasm. Hertwig (19, p. 13) speaks of protoplasm as a 'viscous, almost always colourless, in water-immiscible substance', and Bütschli (7, p. 543) says, 'Since the fact exists . . . that protoplasm is not miscible in water . . .' These statements are probably the outcome of direct observations on the behaviour of protoplasm in water. Such observations can be readily made by aid of microdissection.

If an ovum of the sea-weed *Fucus* or of an echinoderm is torn by microdissection needles, one of three things will happen: the egg will most often heal the wound with little or no loss of protoplasm; occasionally it will burst at the first touch of the needle and the egg contents scatter with the violence of a miniature explosion (37, p. 316); rarely will the protoplasm flow out and mix with the surrounding water. In the last two cases the escaped cell content is miscible, but it is then no longer real living protoplasm, for those peculiar properties which make protoplasm a living substance are lost. If the tear is so large that much of the protoplasm escapes, this escaping protoplasm will usually round up into droplets with no indication of mixing with the water. The dissection of many dozens of marine ova supports this statement. To cite but one instance—the dissection of some twenty eggs of the sea-urchin *Tripleneustes* resulted in two cases of rapid miscibility, and these were both of eggs which showed other signs of degeneration such as previous excessive imbibition. The rate and percentage of spoiling among marine ova is rather great. These degenerate eggs go to pieces readily. Frequently, a healthy egg will exhibit great resistance and tolerate a surprising amount of dissection. For example, one *Tripleneustes* ovum withstood several minutes of dissection, during which time the protoplasm was kneaded, torn, and droplets of it pinched off. There was no indication of miscibility until apparently the 'organization' of the protoplasm finally completely broke down, and general disintegration set in, with, of course, loss of individuality of the cell—that is, of those properties which make of the cell an organized living unit.

The same behaviour characterizes the protoplasm of myxomycetes. Plasmodia can be punctured, torn, stretched, droplets pinched off, and fresh surfaces of the peripheral hyaloplasm or the inner granular plasma exposed to the surrounding water, and nineteen times out of twenty the wound is healed.

The same treatment of *Amoeba* will more often result in loss of proto-

plasm. The ectoplasm nearly always maintains its identity, but the very dilute endoplasm frequently mixes with the water when it is exposed by a deep rupture. Here is an exception to the general rule stated above, namely, that protoplasm is immiscible in water. There are, however, three important facts to remember in connexion with this uncommon instance of apparently true miscibility of protoplasm: first, the protoplasm which is miscible is exceedingly dilute; second, the mixing of the liquid endoplasm with water takes place in only about fifty per cent. of the cases; and third, with miscibility the protoplasm as such is lost. The endoplasm of the ciliate infusorium *Euplates*, which is the most liquid protoplasm of any examined by me, is also frequently miscible in water. Escaped protoplasm from pollen tubes is another exception to the general rule that protoplasm is immiscible. But here, likewise, the protoplasm is very dilute and mixing takes place in only about half the number of cases.

In examining protoplasm with especial reference to its miscibility in water one must not be misled by the miscibility of an abundance of cell sap, which is not strictly protoplasm and which occupies so large a part of the interior of some plant cells. If one of the large internodal cells of *Chara* be cut in two the contents will flow out and diffuse rapidly in the surrounding water, but the by far greater portion of this outflowing substance is cell sap. The *Chara* protoplast consists of two thin layers (the outer quiescent, the inner streaming) of protoplasm lining the cell wall, and the bulk of the cell content is made up of the vacuolar cell sap. It is this cell sap which mixes in the surrounding water, while what little of the protoplasm flows out can be seen as small isolated and immiscible clumps scattered about in the water.

The above experimental facts justify the conclusion that living protoplasm, in the great majority of instances, and in all cases where the living substance is normal and above a minimum viscosity, is immiscible in water.

Whatever our point of view on the water-miscibility of protoplasm, we are forced to admit that in most instances it does not mix with water when exposed to it. The question now arises, Why is protoplasm usually immiscible? Chambers (9, p. 2), who holds that protoplasm is miscible in water, answers this question by saying that the condition of miscibility obtains *unless* a protective membrane or surface layer intervenes. Viewed in this light the problem practically becomes non-existent, because in most instances a membrane *does* intervene, and when no membrane intervenes we are usually justified, for other reasons than the inability of the protoplasm to form a membrane, in assuming the protoplasm to be degenerate. That the surface layer is in many respects protective may be reasonably assumed, but is not the surface layer, protoplasm? It differs, to be sure, physically, and possibly also chemically, from the inner protoplasm, just as the ectoplasm differs from the endoplasm, and the endoplasm on the one side of a cell may differ at any moment from the endoplasm on the other side of a cell.

There is another method of attack on this problem, namely, by water injection. It is this method which probably gives Chambers his chief evidence of the miscibility of protoplasm. He (9, p. 2) says, 'If a drop of water be injected slowly and gradually into the egg by means of the mercury injection method, the water diffuses throughout the cytoplasm, diluting it'. Thus also will a blotter or sponge take up water if it is slowly fed to it. Injected sea-water is, however, not always taken up by the protoplasm. Frequently a droplet of water is held in the ovum for some time. The only miscibility of protoplasm which results is the dilution of a small amount of disorganized protoplasm injured by the injection. Chambers (9, p. 12) attributes the formation of a water droplet to 'mechanical compression caused by the force of the injection', which produces 'a coagulation film about the injected droplet to form a vacuole'. There would thus obtain a condition identical with that of the surface protoplasm of myxomycetes or *Amoeba*. The membrane of the protoplasm surrounding such an injected water droplet exhibits osmotic properties similar to those of the exterior plasma-membrane, as shown by the experiments of Kite (22, p. 4), who says, 'A small dose of distilled water is taken up by the surrounding cytoplasm of the starfish egg quite slowly. A vacuole of sea-water requires a somewhat longer time to disappear, while a vacuole filled with hypertonic sea-water increases in size.'

Injection of water does not always result in the formation of a water vacuole. If the quantity injected be sufficiently great, most of the protoplasm goes into solution, i.e. is truly miscible. Some of the egg contents may remain undiluted. What have we as a result of such a dilution? It requires but a glance or a tear with a needle to demonstrate the degenerate state of the protoplasm. Normal living protoplasm as such no longer exists.

Dead protoplasm is frequently water-miscible. The degenerate coagulum usually disintegrates, sometimes slowly and sometimes with great rapidity, though it may persist in water for hours. The rate of diffusion may even vary in different regions of the same protoplasmic mass (37, p. 316).

Miscibility apparently results from a break-down of organization. Excessive imbibition and consequent swelling of a *Fucus* ovum before diffusion of the egg contents suggests this. With a break-down of organization, which may be nothing more than a collapse of colloidal structure, the protoplasm is no longer able to resist imbibition pressure, and diffusion results.

Two main factors, then, seem to be responsible for the miscibility of protoplasm, namely, disorganization (in by far the greater number of cases) and extreme liquidity (in some few instances).

A very interesting instance of protoplasm seemingly 'attempting' to

hold its own against disorganization is the following observed behaviour of the escaped protoplasm of a sea-urchin ovum. I have watched such free protoplasm go through the process of rounding up, which is, when the protoplasm is very viscous, comparatively slow. Usually the process of healing is completed and the escaped droplet of protoplasm maintains its identity. Often, however, the droplet is not quite fully formed, part of its surface remaining momentarily ragged. This exposed, ragged protoplasm, without any apparent membrane, remains, until the turning-point in the process is reached, quite immiscible in the water with which it is in contact. One can almost imagine the protoplasm 'struggling' to retain itself. If it succeeds, the droplet is formed. If it fails, miscibility results, as a consequence of disorganization.

To deny the water-miscibility of a hydrosol forces one to explain an apparent incongruity. But one must bear in mind that protoplasm is not a simple emulsion of two phases such as milk, but is exceedingly complex, possessing an intricate colloidal structure and a still more intricate and as yet unfathomable organization.

MacDougal (25, p. 601) has suggested that solubility of protoplasm may depend 'upon differences in the carbohydrate component', i.e. 'living matter in which the pentosan was a mucilage like gum arabic would be miscible with water, while a pentosan like tragacanth would be less soluble, and a group like agar, for example, would not appear to be soluble at all'. Solubility 'might also result from the character of the amino-compounds or proteins present, especially in a protoplasm rich in nitrogen'. This does not, it seems to me, explain the true state of affairs. It is probably structure primarily which keeps protoplasm from losing its identity as living substance by mixing in the surrounding water. I do not mean to imply that structure alone is the deciding factor, for chemical changes may conceivably play at least a minor part. For example, oil, through the activity of an enzyme, may be split into glycerine and an acid and thus become soluble in water. But the emphasis in the case of protoplasm should, I think, be placed upon structure rather than upon chemical composition.¹

Gaidukov (16, p. 161) says that 'protoplasm can only be a hydrosol if it is covered by a protective wall (plasma-membrane)'. It is, I grant, difficult to conceive of a hydrosol being immiscible in water unless a membrane intervenes. That a membrane does usually intervene is true, but it is itself protoplasm and as such gives no evidence, even when liquid, of miscibility. The membrane is apparently not the cause but a result of

¹ It is possible that the large size of the molecules of the components of protoplasm is a factor in preventing miscibility. The diffusion rate of a solute varies directly as the molecular energy (or the absolute temperature) and inversely as the viscosity of the liquid and dimensions of the particles (25, p. 1051). The protein molecules which make up a large part of protoplasm are among the largest known, consequently they diffuse very slowly.

immiscibility of the protoplasm. Perhaps it is only the true sol state of protoplasm (the endoplasm of *Amoeba*, e.g.) which is sometimes water-miscible, and only when the liquid endoplasm rapidly attains a higher viscosity at its exposed surface does it save itself from destruction. This hypothesis would demand a gel state of protoplasm even when the living substance is of quite low viscosity. Whatever our point of view we have the experimental fact that living, normal protoplasm does not mix with water, unless *extremely* dilute, and even then it does so in only half the cases observed.¹

The affirmation of the miscibility of protoplasm in water robs, it seems to me, the living substance of all organization, robs it of its innate power to maintain its individuality, and makes of this highly complex colloid a comparatively simple aqueous dispersion which can be mixed in water with no change other than a dilution. In view, then, of these facts, how are we to interpret the taking up of water by protoplasm?

The taking up of water by protoplasm is, in my opinion, essentially an imbibition process. The colloidal constitution of protoplasm is responsible for the absorption and retention of water by the living substance.

SUMMARY.

1. Protoplasm when subjected to dissection in water is, in by far the greater number of instances, immiscible in the surrounding medium.
2. When the condition of water-miscibility of protoplasm does occur, it is ascribable to one of two factors—extreme liquidity or disorganization (i. e. death).
3. The immiscibility of protoplasm is possibly due to the characteristic colloidal nature of the living substance, which may be merely colloidal structure, although chemical constitution is probably also a controlling factor.
4. The absorption and retention of water by protoplasm is essentially an imbibition process.

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¹ In connection with the problem of protoplasmic miscibility it is interesting to consider the protrusion of 'naked' protoplasm through clefts and pores in diatoms. It is difficult to conceive of such an external stream of protoplasm, by means of which diatoms are supposed to move (33, p. 118¹) as water miscible.

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NOTES.

NOTE ON THE PRESENCE OF A 'TENT-POLE' IN THE SEED OF CEPHALOTAXUS PEDUNCULATA.—In a recent paper published in the 'Annals of Botany' the view was expressed that, apart from *Ginkgo*, the nearest known relatives of the genera *Taxus*, *Torreya*, and *Cephalotaxus* are to be sought among the Palaeozoic group Cordaitales. The relatively isolated position of the three genera has also been urged in another paper,² in which the suggestion has been ventured that in view of their structural differences from the great majority of Conifers, coupled with certain archaic features reminiscent of Palaeozoic times, these three genera deserve to rank as an independent phylum (Taxales) co-ordinate with the Ginkgoales and the Coniferales (in the restricted sense). Like *Ginkgo biloba*, in fact, the Taxales were claimed as 'links with the past'.

The object of the present note is to draw attention to another fact which appears considerably to strengthen the Cordaitalean affinity. This is the presence, in the seed of *Cephalotaxus pedunculata*, of a small apical prolongation of the female prothallus

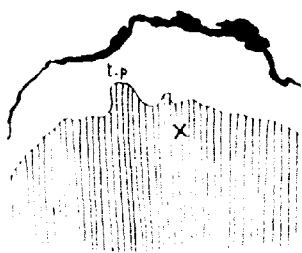


FIG. 1. *Cephalotaxus pedunculata*.
t.p. = tent-pole. x = position of proembryo.

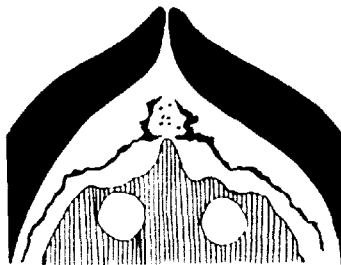


FIG. 2. *Cycas argentea*.
Modified from Renault.

which, surrounded by a moat-like depression into which the archegonia open, props up the nucellar membrane somewhat like a 'tent-pole'. (The comparison of this process to a 'tent-pole', first made by Hirasé³ while describing it in *Ginkgo*, is so appropriate that this convenient term may usefully be adopted as a technical name.) This apparently insignificant organ of obscure function has long been known as a notable point in the structure of the few petrified seeds that have been found

¹ Sahni, B.: On Certain Archaic Features in the Seed of *Taxus*, &c. Ann. Bot., xxxiv, 131, 1920.

² Phil. Trans. Roy. Soc., ccx. 253, 1920.

³ Hirasé, S.: Études sur la fécondation et l'embryogenie du *Ginkgo biloba*. Journ. Coll. Sci., Imp. Univ., Tokyo, 1895.

attached to Cordaitan shoots. The same feature is to be observed with suspicious constancy in a number of detached Palaeozoic seeds which have for good reasons been attributed to the Cordaitales, and it may safely be regarded as a strong Cordaitalean characteristic.¹

Fig. 1 is from a longitudinal section prepared from a seed of *Cephalotaxus pedunculata* after removal of the integument and all but the apical region of the female prothallus. Fig. 2 represents the corresponding parts (and a portion of the integument) in *Cycadinocarpus angustodunensis*; it has been inserted to facilitate a comparison between the recent and fossil seeds. A fairly well developed tent-pole is seen in Fig. 1; the position of the archegonia corresponds with that in Fig. 2, but at the stage examined a proembryo had already been formed in the position marked by a cross.

The presence of a tent-pole in *Cephalotaxus pedunculata* naturally suggests that this organ should be looked for elsewhere in the group. In 1905 Coulter and Land² published a figure of *Torreya taxifolia*, Arnott, in which the female prothallus shows a very striking cone-like apex: it would be interesting to know whether this may be homologized with a tent-pole. It is not unlikely that a search for this organ may prove successful in other members of the group.

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December 14, 1920.

DIVISION OF THE NUCLEI IN SYNGHYTRIUM ENDOBIOTICUM, PERC.

—The life-history and mode of infection of *Synchytrium endobioticum*, a problem which has baffled a number of previous students, has recently been fully investigated by K. M. Curtis ('Phil. Trans. Roy. Soc.,' Ser. B, vol. ccx, p. 409).

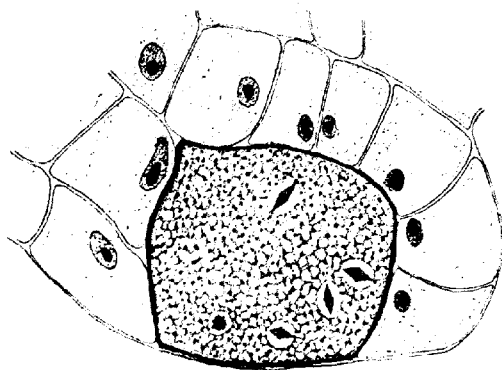
The protoplasm of the resting sporangium of this organism is unusually dense, being crowded with deeply staining granules, and Curtis was unable to observe mitosis in connexion with the development of zoospores in the sporangium although mitosis was clearly recognized during the corresponding stages in the sorus. She was led to the conclusion that in the resting sporangium the primordia of the zoospores arise from chromatic granules discharged by the primary nucleus.

While making microscopic observations of this organism, in connexion with the problem of immunity, I had recently occasion to investigate the effect of various fixatives; among these Perenyi's fluid was employed and was found, probably owing to its poor fixation, to leave the normally dense cytoplasm in a transparent condition. As a result I was so fortunate as to be able to observe very definite mitoses in a developing resting sporangium which showed six dividing nuclei. Five of these are represented in the accompanying figure, the sixth was found in the adjacent

¹ See the works of Ad. Brongniart and of C. E. Bertrand cited in my paper on *Taxus* above referred to. Also Seward, Fossil Plants, iii, 333, 1917; Coulter and Chamberlain, Morphology of Gymnosperms, p. 197.

² Coulter and Land: Gametophytes and Embryo of *Torreya taxifolia*. Bot. Gaz., XXX, Pl. A, Fig. 5, 1905.

section. The spindles are developed apparently within the nuclear membrane, and there is possibly some indication of the presence of centrosomes. The chromosomes are small and are crowded on the equatorial plate and could not be counted. There



Semi-diagrammatic drawing showing mitosis in resting sporangium of *Synchytrium endobioticum*, Perc. $\times 600$.

is no reason to doubt that in the resting sporangium, as in the sorus, the zoospore nuclei are the product of indirect division.

As circumstances have made it impracticable for me to continue the work in question it has seemed worth while to place this single observation on record.

E. J. WELSFORD.

IMPERIAL COLLEGE OF SCIENCE,
January, 1921.

The Leaf Structure of the Iridaceae, considered in Relation to the Phyllode Theory.

BY

AGNES ARBER, D.Sc., F.L.S.,

Kedley Fletcher-Warr Student of the University of London.

With sixty-six Figures in the Text.

CONTENTS.

	PAGE		PAGE
1. INTRODUCTION	301	4. LEAVES OF THE Iridoideae AND PHYLLODES OF <i>ACACIA</i>	317
2. ENSIFORM PETIOLAR LEAVES	303	5. FIBROSIS IN IRIDS AND ACACIAS	319
(i) The occurrence of ensiform leaves in the Iridaceae	303	6. FOLIATED LEAVES IN THE Iridoideae AND Ixiodeae	320
(ii) Views hitherto held on the nature of the ensiform leaf	303	7. THE LEAVES OF THE Crocoideae	323
(iii) The ensiform leaf as a petiolar structure, and the comparison with <i>Acacia</i> phyllodes	303	8. ASYMMETRY IN CERTAIN IRID LEAVES	328
(iv) Semi-equant leaves	306	9. THE EVOLUTIONARY HISTORY OF THE IRID LEAF	329
(v) The association of ensiform leaf and winged axis in certain Monocotyledons and Acacias	308	(i) The primitive character of the ensiform leaf-type	329
3. NON-ENSIFORM LEAVES IN CERTAIN Iridoideae	310	(ii) The progression from the ensiform leaf-type	331
(i) 'Radial' leaves in <i>Iris</i> and other Iridoideae, and their relation to <i>Acacia</i> phyllodes	310	10. SUMMARY	333
(ii) Transitional and bifacial leaf-types in <i>Iris</i> and <i>Moraea</i>	312	ALPHABETICAL LIST OF MEMOIRS CITED	335

1. INTRODUCTION.

THE present paper forms one of a series¹ in which I am attempting to test the validity of the phyllode theory by studying the results of its application to the leaves of various groups of Monocotyledons. In this instalment I propose to deal with the Iridaceae—that family in which, on my view, the phyllodic leaf finds perhaps its most varied and complete

¹ Arber, A. (1918), (1919¹), (1920¹), (1920²), (1920³).

[Annals of Botany, Vol. XXXV, No. CXXXIX, July, 1921.]

expression. The thesis which I hope here to substantiate is that *the leaf of the Iridaceae has no true lamina, but represents, in every case, either a petiole and leaf-base, or a leaf-base alone.*

A considerable body of work on the leaf anatomy of the Irids already exists,¹ that of the Italian writer, Hermann Ross, being particularly thorough and detailed. But I have found that, since none of the botanists who have hitherto dealt with the subject have even considered the possibility of the leaf in question being merely a modified leaf-base and petiole, their observations seldom include answers to the particular questions to which the phyllode theory gives rise. I have, therefore, found it necessary to attempt an independent examination of the principal leaf-types met with in the Iridaceae. In this connexion I must express my gratitude, for their kindness in supplying me with material, to the Director and to the Keeper of the Herbarium, the Royal Botanic Gardens, Kew, and to the Keeper of the Department of Botany, British Museum (Nat. Hist.), and also to Professor Sir Isaac Bayley Balfour, F.R.S., Professor Béguinot, of Padua, Mr. Joseph Benbow, of La Mortola, the late M. Augustin de Candolle, of Geneva, Mr. F. J. Chittenden, Miss Mabel Cobbe, Mr. W. R. Dykes, Mr. C. C. Lacaita, Miss C. E. Larter, Mrs. J. J. Lister, Mr. R. I. Lynch, Mr. J. H. Maiden, F.R.S., of Sidney, Dr. Schonland, of Grahamstown, and Professor A. C. Seward, F.R.S. I am much indebted to Mr. W. R. Dykes and to Professor J. Bretland Farmer, F.R.S., for suggestions and criticism, and to Miss E. R. Saunders for facilities for carrying out this work in the Balfour Laboratory, Cambridge.

Regarding the classification of the Iridaceae there is considerable difference of opinion; in the present paper I have provisionally adopted the scheme used in Engler's 'Pflanzenfamilien'.²

2. ENSIFORM PETIOLAR LEAVES.

(i) *The occurrence of ensiform leaves in the Iridaceae.*

In a previous paper³ I have briefly discussed the ensiform (or isobilateral equitant) leaf, and its relation to other phyllodic types. This form of leaf—the most widespread of those met with amongst Monocotyledons which show the type of anatomy that I have interpreted as phyllodic—is characteristic of the majority of the Iridaceae;⁴ it occurs in a large proportion of the Iridoideae and Ixioidae, though not in the Crocoideae. In examining the leaves of the family in the herbarium of the British Museum (Nat. Hist.) I have seen ensiform leaves in the following genera: *Acidanthera*, *Antholyza*, *Aristea*, *Belamcanda*, *Bobartia*, *Crocodynia*,

¹ Chodat, R., and Balicka-Iwanowska, G. (1892); Balicka-Iwanowska, G. (1892-3); Ross, H. (1892-3); Lindman, C. A. M. (1899).

² Pax, F. (1888).

³ Arber, A. (1918), p. 482.

⁴ Ross, H. (1892-3).

Cypella, Dierama, Diplarrhena, Freesia, Geissorhiza, Gladiolus, Hesperantha, Iris, Ixia, Lapeyrousia, Libertia, Marica, Melasphaerula, Micranthus, Moraea (Diets), Patersonia, Schizostylis, Sisyrinchium, Sparaxis, Synnotia, Tapeinia, Tritonia, Watsonia, Witsenia. The prevalence of the ensiform leaf, indicated by the length of this list (which is not exhaustive), seems to justify us in opening our consideration of the Iridaceae with a renewed attempt to understand this foliar type, which has been the subject of much controversy.

(ii) *Views hitherto held on the nature of the ensiform leaf.*

The anomalous character of the equitant *Iris* leaf has long been recognized; in Lyte's 'Herball' of 1578 it is compared to 'the blade of a two-edged sworde'. It is one of the most familiar examples of the 'monofacial' leaf as opposed to the more usual flattened dorsiventral type. According to Čelakovský,¹ Velenovský,² and others,³ the ensiform leaf arises through congenital concrescence. This view has been most thoroughly and consistently elaborated by Čelakovský, who follows out and accepts all the deductions that arise logically out of his theory. He holds that the bifacial leaf is universally primary, and that all monofacial leaves are produced by concrescence of the halves, to right and left of the midrib, which were originally free and flat. He even goes so far as to suppose that all petioles came originally into existence through congenital folding and fusion of the narrowed basal region of bifacial blades. Velenovský, however, while accepting Čelakovský's view for the ensiform leaf, and even for those species of *Iris* which have a 'radial' limb, refuses to apply it to such apparently similar monofacial leaves as those of *Juncus communis*, which he interprets as due to the thickening of an originally flat blade. Goebel,⁴ on the other hand, takes a view entirely opposed to those of Čelakovský and Velenovský. He declines altogether to accept the idea of congenital concrescence, but he gives no explicit statement of his opinion as to the morphological nature of the limb. He treats it as a new wing-like outgrowth from the original leaf primordium, and, as far as one can judge from the way in which he discusses it, he seems to regard it as an organ *sui generis*.

(iii) *The ensiform leaf as a petiolar structure, and the comparison with Acacia phyllodes.*

In my 1918 paper⁵ I have put forward the view that the equitant leaf which characterizes the Irises belonging to the Sections *Apogon*, *Pogoniris*,

¹ Čelakovský, L. J. (1903).

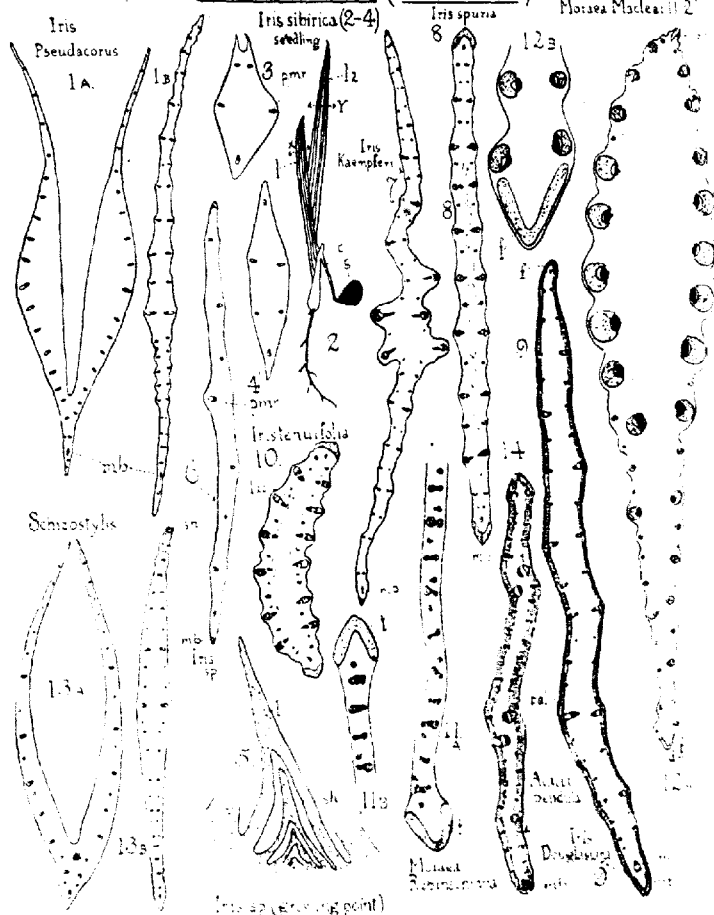
² Velenovský, J. (1907).

³ Gray, Asa (1867); Chodat, R., and Balicka-Iwanowska, G. (1892); Balicka-Iwanowska, G. (1892-3); &c.

⁴ Goebel, K. (1905 and 1913).

⁵ Arber, A. (1918); Candolle, A. P. de (1927), recognized the leaf of *Iris* as petiolar.

Mazaea Midea: 12'



FIGS. 1-14. (All figures in this paper, unless otherwise stated, are drawings of transverse sections of limbs of leaves, with the adaxial side towards the top of the page; xylem, solid black; phloem, white; fibres, dotted; *m.b.*, median bundle.) Fig. 1, *Iris Pseudacorus*, L. (x 7); only principal bundles indicated; Fig. 1 A, sheath; Fig. 1 B, limb. Fig. 2, *Iris sibirica*, L. Fig. 2, seedling (nat. size); *s*, seed; *c*, cotyledon; *l*₁ and *l*₂, first and second plumar leaves. Fig. 3, transverse section of *l*₁ at *x* (x 23). Fig. 4, transverse section second leaf of another seedling at about level *y* in *l*₂ (x 23). Fig. 5, *Iris* sp., longitudinal section stem apex with young leaves; *l*₁, solid limb; *sA*, sheath (x 14). Fig. 6, *Iris* sp., China (x 14). Fig. 7, *Iris Kaempferi*, Sieb. (x 7). Fig. 8, *Iris spuria*, L. (x 14). Fig. 9, *Iris Douglasiana*, Herb. (x 14), the palisade parenchyma, *pal*, more developed on left-hand face. Fig. 10, *Iris tenuifolia*, Pall. (x 14); *lac*, lacuna crossed by trabeculae. Fig. 11, *Moraea Robinsoniana*, C. Moore et F. Muell. (x 14). To economize space the section is drawn in two parts, A and B; *f*, fibres. Fig. 12, *Moraea Macleanii*, Hort.; Fig. 12 A, limb at extreme top of junction with sheath, *sA*; *d.m.*, dorsal margin; *f*, fibres (x 14); Fig. 12 B, dorsal margin of Fig. 12 A (x 47). Fig. 13, *Schizanthyllis coccinea*, Backh. et Harv. (x 7); Fig. 13 A, sheath; Fig. 13 B, limb. Fig. 14, *Acacia pendula*, A. Cunn. (x 14).

&c., did not arise by congenital concrescence, but is best regarded as a petiolar phyllode, comparable with a vertical *Acacia* phyllode, from which it differs in possessing a sheathing base. I tried to show that this view is supported by a study of the external form and internal structure, by a comparison with other 'monofacial' types, especially those met with in the Iridaceae, and also by the facts of the ontogeny as described by Goebel and Trécul. Since that paper was written, I have examined the apical bud of an *Iris* with ensiform leaves, and I have found that by dissecting it under the simple microscope I could satisfy myself that the leaves, down to one which was less than 1 mm. in its greatest dimension, showed a solid upper region distinct from the sheathing base. Fig. 5 represents a section from a microtome series passing longitudinally through a similar apical bud, in which the same distinction of solid terminal limb (*l.*) and sheathing base (*sl.*) can be recognized even in leaves whose youth is extreme. The ontogeny thus yields no evidence at all for congenital concrescence. The same is true of the seedling structure (Fig. 2), for the solid phyllodic character is already recognizable even in the first plumular leaves (Figs. 3 and 4).

Figs. 1-10 illustrate the general anatomy of various ensiform *Iris* leaves. In *Iris Pseudacorus*, L. (Figs. 1 A and B), and *I. spuria*, L. (Fig. 8), the bundles on the two sides of the leaf are equal and opposite; in *I. Kaempferi*, Sieb., which is conspicuously ribbed (Fig. 7), and *I. Douglasiana*, Herb. (Fig. 9), the bundles on either side are found to be unequal in size and somewhat irregularly disposed, while in an undetermined species from China (Fig. 6) the bundles alternate. I also include here a sketch of *I. tenuifolia*, Pall. (Fig. 10), because it has been figured by Chodat and Balicka-Iwanowska¹ as lacking a median bundle, whereas I find it to be of the normal *Iris* type in this respect.

The ensiform leaves of *Moraea Robinsoniana*, C. Moore et F. Muell., and *M. Macraei*, Hort. (Iridoideae), are represented in Figs. 11 and 12. Figs. 13 A and B show the leaf structure in the Kaffir Lily, *Schizostylis coccinea*, Backh. et Harv. (Ixioidae), which is essentially similar to that of *Iris Pseudacorus*.

In my previous paper² the phyllodes of *Acacia nerifolia*, A. Cunn., *A. scirpifolia*, Meissn., and *A. Cyclops*, A. Cunn., were figured and compared with the leaves of various Iridaceae; a further study of *Acacias* has shown that this comparison is even more widely applicable than was then supposed. Fig. 14 illustrates the structure of the phyllode of *Acacia pendula*, A. Cunn., which may be compared with the ensiform leaves of the genus *Iris*, especially with *I. Douglasiana* (Fig. 9). Figs. 40-4, p. 318, demonstrate the relation of *Sparaxis*, and of two ensiform species of

¹ Chodat, R., and Balicka-Iwanowska, G. (1922), Fig. 10, p. 258.

² Arber, A. (1918), Figs. 1 A and B, 2 A-D, p. 474, and Fig. 21, p. 483.

Gladiolus, to *Acacia neurophylla*, W. V. Fitz, and *A. uncinella*, Benth.; Figs. 45 and 46 show the close resemblance between *Gladiolus ornatus*, Klatt, and *Acacia incurva*, Benth., while Figs. 27 and 28, p. 311, indicate the identity in type of *Sisyrinchium junceum*, E. Mey., and *Acacia teretifolia*, Benth. The similarity of these Irid and *Acacia* leaves seems to me to supply a strong argument against the congenital concrescence view. There can obviously be no question of concrescence in *Acacia*, since here we have no sheathing base, and thus the limb cannot possibly be interpreted as arising through the fusion of the margins of such a sheath. Yet the parallelism between the very various types met with among *Acacia* phyllodes, and many of the Irid leaf forms, is too exact in all essentials to be treated as fortuitous; it appears far more likely that the same morphological interpretation applies to both. The evidence of the leaf succession in the seedling makes it certain that in many *Acacias* the leaf of the mature plant is a petiolar phyllode, and the balance of probability favours the idea that the Irid leaf should come under the same category.

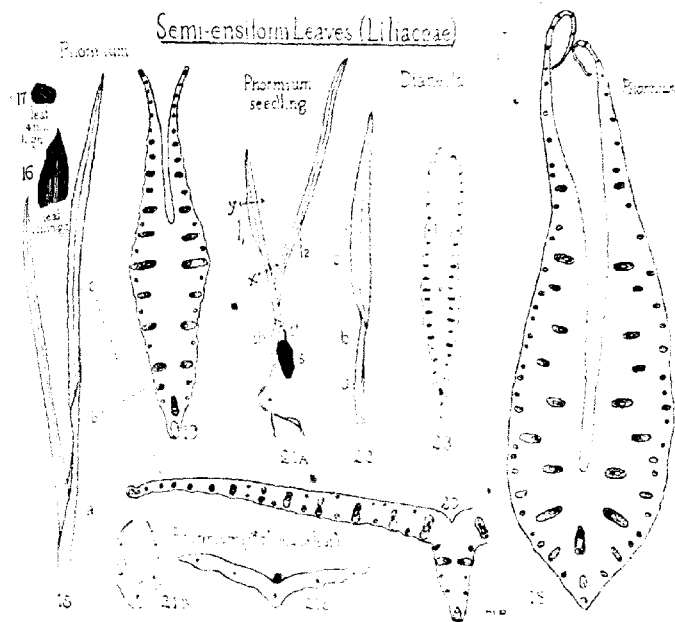
(iv) *Semi-equitant leaves.*

In a previous paper¹ I drew attention, very briefly, to the case of the Liliaceous plant, *Phormium tenax*, Forst., the New Zealand Flax; further work has inclined me to an alternative interpretation of the curious leaf structure of this plant—an interpretation which I think is more likely to prove valid than the view I tentatively suggested in 1918. In *Phormium* and the related genus *Dianella*, there is, in the mature leaves, a sheathing base (*a* in Figs. 15 and 22) and a normal bifacial limb (*c* in Figs. 15 and 22), but between these parts there is a short, vertically flattened region (*b* in Figs. 15 and 22) whose structure and anatomy (Figs. 19 and 23) recall the limb—or rather the transition region between sheath and limb—in the ensiform species of *Iris*. In consequence of this peculiarity, Velenovský² describes the leaves of *Phormium* and *Dianella* as 'semi-equitant', and claims them as an obvious case of congenital concrescence. To test his view I have looked into the ontogeny of the leaf, which I find to be wholly different from that of *Iris*. The very young leaves are entirely open and sheathing (Figs. 16 and 17), and this bifacial stage, in which there is no vertical ensiform region, continues until the leaves have reached a considerable size. For instance, in a leaf a little more than 30 cm. long, the closed vertical region was found to be just beginning to differentiate itself from the sheathing base. In the seedling, also, the first plumular leaves are entirely open and sheath-like (Figs. 21 A-C). The evidence from ontogeny and from seedling structure thus appears definitely to support Velenovský's contention that we have here a genuine instance of congenital

¹ Arber, A. 1915.

² Velenovský, J. (1907).

concrecence. But the same line of evidence seems to me to indicate that the leaf of *Iris* is wholly different in its morphology from those of *Phormium* and *Dianella*, and that it does not, as Velenovský assumes, represent a case in which the congenital concrecence of *Phormium* has been carried to an



FIGS. 15-23. FIGS. 15-21, *Phormium tenax*, Forst. FIG. 15, two of the younger leaves of shoot ($\times 1$); the left-hand leaf is open to the base, with no concrecent region; the right-hand leaf has an open sheath at *a*, a concrecent region at *b*, and a flat limb at *c*. FIG. 16, youngest leaf of shoot in Fig. 15, open to base (slightly enlarged). FIG. 17, leaf 4 mm. high from another shoot, open to base (slightly reduced). FIG. 18, transverse section at *a* across sheathing region of right-hand leaf in Fig. 15 ($\times 9$, circa). FIG. 19, transverse section at *b* across concrecent region of right-hand leaf in Fig. 15 ($\times 9$, circa). FIG. 20, transverse section at *c* (incomplete) across limb region of right-hand leaf in Fig. 15 ($\times 9$, circa). FIG. 21 A-C, seedling; FIG. 21 A, seedling ($\times 1$); *l*, 1st leaf; *l*, 2nd leaf; *sh*, sheath of cotyledon; *st*, stalk of cotyledon; *s*, seed; FIG. 21 B, transverse section of *l* in Fig. 21 A, at level *x* ($\times 15$, circa); FIG. 21 C, transverse section of *l* in Fig. 21 A at level *y* ($\times 15$, circa). FIGS. 22 and 23, *Dianella nemorosa*, Lam. FIG. 22, leaf with sheathing region, *a*, concrecent region, *b*, and open limb, *c* (reduced). FIG. 23, transverse section of leaf in Fig. 22 at level *b*, where fusion is most complete ($\times 9$, circa).

extreme point. I look upon *Phormium* and *Dianella* as plants in which a partially 'pseudo-phyllodic' leaf structure and anatomy have been reached as a secondary modification. These two Liliaceous genera would thus be best treated at present as a case apart. It is possible that certain other 'monofacial' leaves among the Monocotyledons may also prove, on further study, to be comparable with those of *Phormium* and *Dianella*.

(v) *The association of ensiform leaf and winged axis in certain Monocotyledons and Acacias.*

A peculiarity which, in many cases among the Iridaceae, is associated with an equitant leaf, is the winging of the aerial axis in the plane of flattening of the leaf limb. In the herbarium of the British Museum (Nat. Hist.) I have observed this winging in the following genera and species:—

Aristea alata, Baker; *A. anceps*, Eckl.; *A. cladocarpa*, Baker; *A. compressa*, Buching.

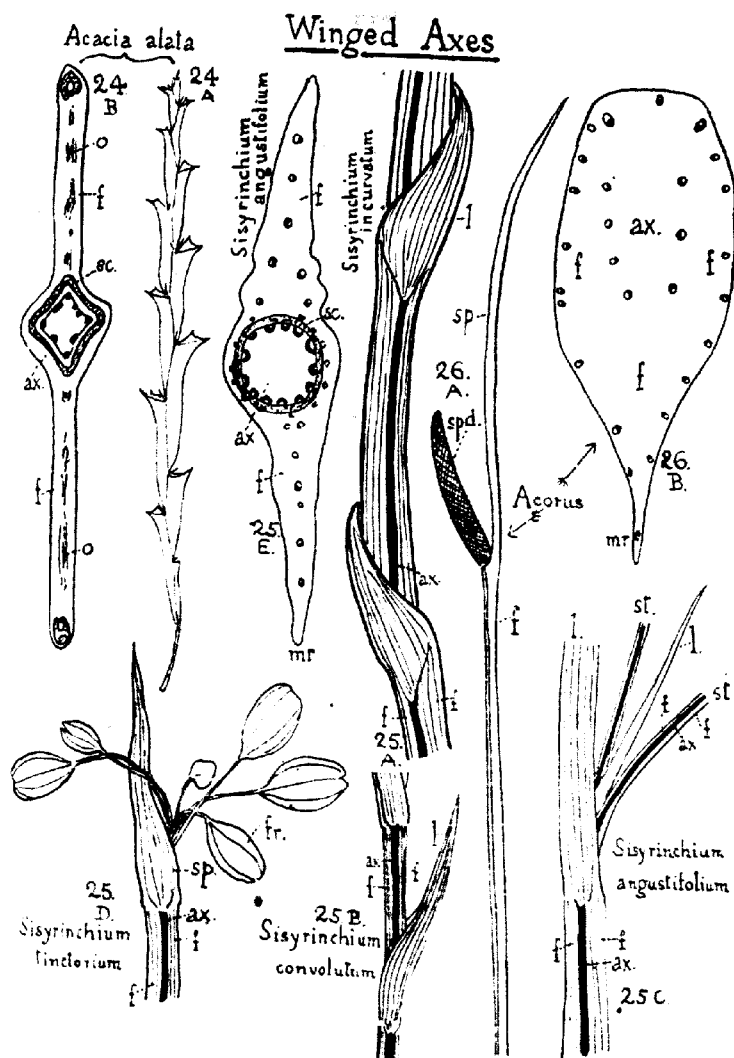
Bobartia gladiata, Ker-Gawl.

Lapeyrussia abyssinica, Baker; *L. compressa*, Pourr.

Marica caerulea, Ker-Gawl.; *M. gracilis*, Herb.; *M. Northiana*, Ker-Gawl.

Sisyrinchium acre, H. Mann; *S. alatum*, Hook.; *S. angustifolium*, Mill. (Figs. 25 C and E); *S. arizonicum*, Roth; *S. bogotense*, H. B. et K.; *S. californicum*, Dryand.; *S. chilense*, Hook.; *S. convolutum*, Nocca (Fig. 25 B); *S. distantiflorum*, Kränzl; *S. grande*, Baker; *S. hirsutum*, Baker; *S. incurvatum*, Gardn. (Fig. 25 A); *S. iridifolium*, H. B. et K.; *S. micranthum*, Cav.; *S. palmifolium*, L.; *S. restioides*, Spreng.; *S. tinctorium*, H. B. et K. (Fig. 25 D); *S. vaginatum*, Spreng.

When the winging is examined in detail, it is found that the leaf-base is continued downwards on either side of the true axis, from which it often remains conspicuously distinct (e.g. *Sisyrinchium incurvatum*, Gardn., Fig. 25 A). This winging is chiefly to be observed in inflorescence axes, since these are frequently the only aerial stems produced by the plants of this family. In such cases it is the spathe below the inflorescence which is continued downwards to form the wing (e.g. *Sisyrinchium tinctorium*, H. B. et K., Fig. 25 D). The sharp distinction between the cylindrical axial region (*ax.*) and the flattened foliar region (*f.*) is very noticeable in the anatomy, e.g. *Sisyrinchium angustifolium*, Mill., Figs. 25 C and E. The almost equal development of the wings on the two sides of the axis in this case, is due to the fact that the spathe forms a closed sheath round the inflorescence for some little distance above its attachment, and its downward continuation is thus capable of forming a wing both on the midrib side and on the side of the fused margins. This case may be contrasted with that of *Acorus Calamus*, L. (Aroideae), Fig. 26 A, in which the spathe is not sheathing, but is connected at the base of the spadix by a very narrow region of attachment. As a result, its downward prolongation produces a wing, but only on the side corresponding to the midrib, *m.r.* (Fig. 26 B).



FIGS. 24-6. Figs. 24A and B, *Acacia alata*, R. Br.; Fig. 24A, shoot (nat. size) to show bases of 2-ranked phyllotides decurrent through two internodes, producing winged axis; Fig. 24B, transverse section winged axis ($\times 28$); ax., axial region; f., foliar region; sc., fibrous hollow cylinder; o., bundles cut obliquely. Figs. 25A-E, *Sisyrinchium*; throughout, ax. = axis (which is indicated in solid black), and f. = foliar wing of stem; Fig. 25A, *S. incurvatum*, Gardn. (nat. size), axis with equitant leaves; f., reduced almost to sheathing bases; Fig. 25B, *S. convolutum*, Nacca (nat. size); Fig. 25C, *S. angustifolium*, Mill., part of branched axis (nat. size); Fig. 25D, *S. tinctorum*, H. B. and K., top of inflorescence axis (nat. size) with spathe, sp., and fruits, fr.; Fig. 25E, *S. angustifolium*, Mill., transverse section winged axis ($\times 28$); mr., continuation of dorsal margin of leaf next above; sc., fibrous hollow cylinder. Figs. 26A and B, *Acorus Calamus*, L.; Fig. 26A, fertile axis, much reduced; spd., spadix; sp., spathe; f., winged side of axis; Fig. 26B, transverse section winged axis below spathe ($\times 7$); mr., continuation of midrib region of spathe. The numerous small bundles lying close to the epidermis are omitted.

The interest of the winged axis, from the standpoint of the phyllode theory, lies in the fact that, just as the main features of the isobilateral equitant leaf of the Irids may be compared with those of *Acacia* phyllodes, so even the minor peculiarity of the winged axis finds a parallel among the phyllodic Acacias. *Acacia alata*, R. Br. (Figs. 24 A and B), has two opposite rows of reduced leaves, whose downward continuation produces a winged axis bearing a general resemblance to that of *Sisyrinchium*, &c., though the occurrence of the wing on the two sides is here due to the fact that each of the two alternating phyllode bases is decurrent through two internodes. Transverse sections show that the parallelism is not confined to mere externals (cf. Figs. 24 B and 25 E); in both cases there is a central axial region (*ax.*)—radial in structure and surrounded by a sheath of fibrous tissue—entirely distinct from the associated lateral wings (*f.*).

3. NON-ENSIFORM LEAVES IN CERTAIN IRIDOIDEAE.

(i) 'Radial' leaves in *Iris* and other Iridoideae, and their relation to *Acacia* phyllodes.

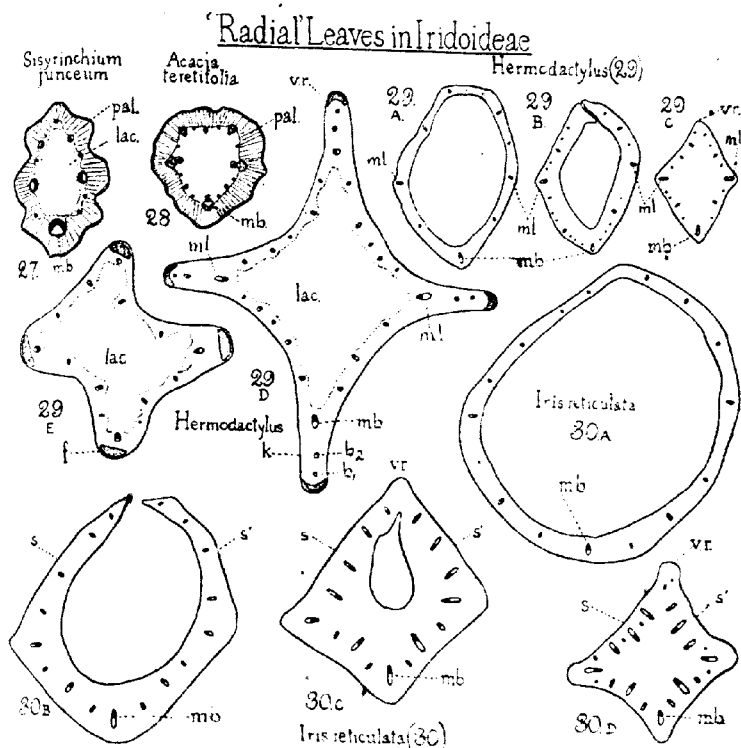
Within the genus *Acacia* we find every transition between phyllodes which are completely flattened in the vertical plane (Figs. 14, p. 304, and 41 and 42, p. 318)¹ and others which are cylindrical and terete (Fig. 28, p. 311).² In various Iridoideae we meet with the same passage from equitant leaves to others that are more nearly radial. The leaves of most Irises of the *Apogon* Section are characteristically ensiform (e.g. *Iris Pseudacorus*, Figs. 1 A and B, and *I. spuria*, Fig. 8), but the genus also includes 'radial' leaves. *Iris tuberosa*, L. (*Hermodactylus tuberosus*, Mill.), is a plant in which the ensiform *Iris* leaf has been modified into a remarkable tetragonal form. Figs. 29 A-C, drawn from a microtome series, show that at the extreme base there is a closed sheath (Fig. 29 A) which opens out a little higher up (Fig. 29 B) and gradually passes into a solid limb, of diamond-shaped section (Fig. 29 C). The median strand (*m.b.*) and main laterals (*m.l.*) occupy three of the four angles, and the ventral margin (*v.r.*) can be distinguished by the fact that it has no bundle with the xylem pointing directly inwards. Higher up, in the mature part of the leaf (Fig. 29 D), we find that each of the four ribs has grown out into a slight keel, supplied as a rule by two small bundles (*b.₁* and *b.₂*) which appear to arise as branches from the main bundles. Close to the apex the fibrous strands, which occupy the angles of the leaf, become increasingly conspicuous (Fig. 29 E).

The bulbous Irises of the *Tetragonae* Section (*I. reticulata*, Bieb., *I. Histrio*, Reichb., *I. Vartani*, Foster, and *I. Bakeriana*, Foster) have

¹ See also Arber, A. (1913), Fig. 2 D, p. 474, and Fig. 21, p. 483.

² Ibid., Fig. 1 B, p. 474.

'radial' leaves, with four to eight angles or ribs, more or less resembling those of *Hermodactylus*. We may take the leaf of *I. reticulata* as an example. In the case of *Hermodactylus* the leaf is fairly symmetrical about a plane passing through the dorsal and ventral margins, but in the



FIGS. 27-30. Fig. 27, *Sisyrinchium junceum*, E. Mey., transverse section limb of leaf; pal., 3-tiered palisade parenchyma ($\times 23$). Fig. 28, *Acacia teretifolia*, Benth., transverse section phyllode; pal., palisade parenchyma of about two tiers of cells ($\times 23$). Figs. 29 A-E, *Hermodactylus tucanus*, Mill.; ml., main lateral; Figs. 29 A-C, sections from microtome series through base of leaf ($\times 14$) showing transition from closed sheath through open sheath to solid limb; vr., ventral ridge; Fig. 29 D, mature part of another leaf; k., keel; b_1 and b_2 , bundles of keel; lac., lacuna; Fig. 29 E, section close to apex of same leaf; f., fibres; lac., lacuna crossed by trabeculae. Figs. 30 A-D, *Iris reticulata*, Bieb., sections from a series through base of limb of leaf ($\times 14$). Fig. 30 A and B, sheath; Fig. 30 C, transition region; Fig. 30 D, limb showing asymmetry of the two lateral faces, s and s' , which meet at the ventral ridge, vr.

case of *Iris reticulata* this is not so. When the leaf is examined *in situ*, the flat surface (s , Fig. 30 D) has all the appearance of being an upper or ventral surface, but Figs. 30 A-D, drawn from a series of transverse sections through a leaf-base, show that the leaf is markedly asymmetrical; the ridge.

v.r., is ventral, while the unequal surfaces, *s* and *s'*, are lateral. Towards the apex the leaf becomes horny; sections show that this horniness is due to a thickening of the epidermal walls.

Sisyrinchium is another genus in which both ensiform and terete leaves occur. Fig. 27 shows the oval ribbed limb of *S. junceum*, E. Mey.;¹ it may be compared with the phyllode of *Acacia teretifolia*, Benth. (Fig. 28). The resemblance is remarkably exact, the vascular strands showing a close approximation in number and distribution.

In the genera *Trimezia* and *Bobartia* the occurrence of cylindrical junciform leaves has also been described.²

(ii) *Transitional and bifacial leaf-types in Iris and Moraea.*

The Irises to which I have hitherto referred are characterized by the unimportance of the leaf-sheath as compared with the limb—at least in the mature leaves—but in the bulbous Irises of the Section *Xiphium*, the sheath takes a more prominent place. In *Iris xiphioides*, Ehr., the English Iris, there is a cylindrical limb (*pet.*), usually of some length (Fig. 31 A), but which may be reduced to a mere trace (Fig. 31 B). I regard such a leaf as a leaf-base (*sh.*) crowned by a petiole, which may be either well developed or vestigial. The evidence on which such a view can be based is set forth in a previous paper,³ in which I have shown that the leaves of certain species of *Tulipa*, *Hyacinthus*, &c., terminate in a cylindrical apex, which by analogy with the transitional forms between the mature leaf and the bud-scale in the Dicotyledon *Fatsia*, I interpret as petiolar. In *Iris xiphioides* we have a leaf of similar morphology, which, however, reveals its nature much more obviously. The apical region of *I. xiphioides* is approximately cylindrical, and its anatomy is so nearly radial that, if transverse sections be taken through it above the sheath, it is impossible to be certain about the orientation from internal evidence. In Fig. 31 D, however, the section passes through the extreme top of the junction of sheath and limb, where the last trace of the sheath cavity, *sh.*, indicates which side is adaxial.

The related species, *Iris Xiphium*, L., the Spanish Iris, is significant because the apical limb is but little modified from the ensiform type (Fig. 32 B). Mr. W. R. Dykes⁴ has pointed out that *Iris spuria* closely resembles *I. Xiphium* in floral characters. It is interesting to find that his idea of a possible relationship between these two species, and, through them, of a connexion between the *Apogon* and *Xiphium* Sections, is confirmed by the evidence of the leaf structure; the limb of *I. Xiphium* (Fig. 32 B) is of a type which would be easily derivable from that of *I. spuria* (Fig. 8, p. 304). The facts of geographical distribution put no difficulty in the way of

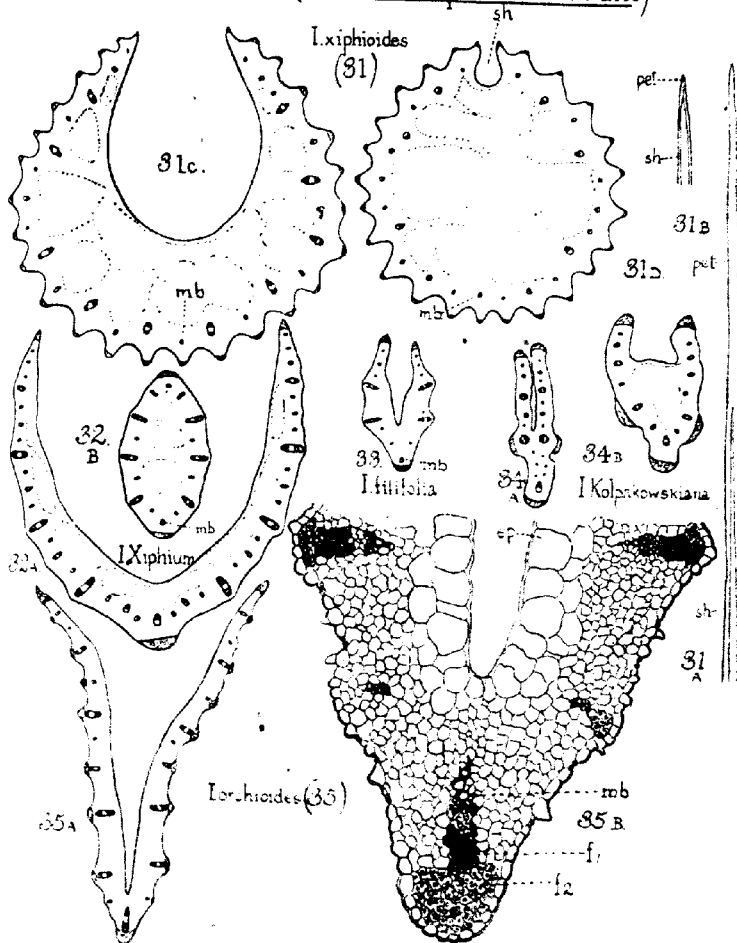
¹ See Arber, A. (1918), Fig. 16 B, p. 483, for a more cylindrical leaf-type belonging to the same genus.

² Ross, H. (1892-3)

³ Arber, A. (1920¹).

⁴ Dykes, W. R. 1913.

Leaves of Iris (Sections *Xiphium* and *Juno*)



FIGS. 31-5. Figs. 31A-D, *Iris xiphioides*, Ehr.: Figs. 31A and B, apical region of two leaves seen from ventral side ($\times 4$) to show variation in length of cylindrical apex (*pet.*) which terminates the bifacial region (*sh.*); Fig. 31C, transverse section bifacial region ($\times 14$); Fig. 31D, transverse section limb at uppermost limit of junction of sheath (*sh.*) and limb ($\times 14$). Figs. 32A and B, *I. xiphioides*, L.: Fig. 32A, transverse section sheath; Fig. 32B, transverse section apical limb; ($\times 14$). Fig. 33, *I. filifolia*, Boiss. ($\times 11$, circa). Figs. 34A and B, *I. kolpakowskiana*, Regel: Fig. 34A, transverse section leaf ($\times 23$); Fig. 34B, transverse section nearer apex ($\times 24$, and B, *I. orchoides*, Carr.: Fig. 35A, transverse section leaf ($\times 14$); Fig. 35B, midrib region of Fig. 35A ($\times 77$, circa). Note contrast between lignified fibres, *f*₁, and non-lignified hypodermal fibres, *f*₂.

accepting Mr. Dykes's view, since Irises of the *Spuria* group are found over the whole range of *I. Xiphium*.

In *Iris filifolia*, Boiss., another of the *Xiphium* Section, the only leaf which I was able to cut had no cylindrical apical region, but it was probably exceptional in this respect.¹ Here, as in the other members of the Section which I have examined, the median bundle is less conspicuous than the main laterals (Fig. 33, p. 313).

The rare species, *I. Kolpakowskiana*, Regel,² whose affinities are somewhat uncertain, is generally associated with the *Xiphium* Section. Its leaf is described as *Crocus*-like in external appearance, but the similarity is in reality quite superficial (contrast Figs. 34 A and B with Fig. 56 I, p. 324, representing the limb of *Crocus Tomasinianus*). The leaf is grooved on the adaxial side to the very apex; it may be regarded as consisting primarily of leaf-sheath, but as including towards the apex the region of transition to the petiolar limb which has itself been lost. It bears a certain resemblance to the dorsiventral region of the leaf of *Iris filifolia*. This resemblance is not, however, very close, and the median bundle is more developed than is usual in the *Xiphium* Section. In this point the leaf rather recalls the Irises of the *Juno* Section. As *I. Kolpakowskiana* is confined to Turkestan, it would seem more probable that its affinities would be with the *Juno* Irises, which range from the Mediterranean region to the North-west Indian frontier, than with the *Xiphiums*, which are restricted to Spain and North-west Africa; but it is evidently a somewhat isolated form.

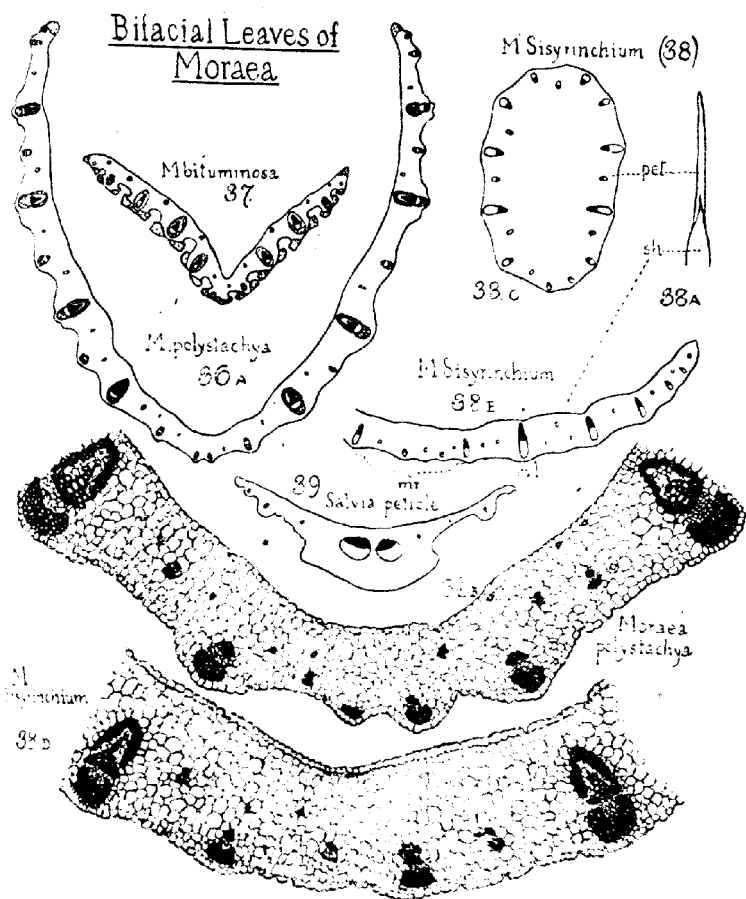
The *Juno* Irises differ from the general *Xiphium* type in being dorsiventral to the extreme apex; that is to say, the leaf is, on my view, more reduced than that of the *Xiphium* Section, and consists merely of leaf-sheath, having lost all trace of the petiole in which it once terminated. The leaves of the *Juno* Irises may be somewhat sharply folded along the midrib, which is, as a rule, at least as important as the main laterals. *Iris orchoides*, Carr. (Figs. 35 A and B) is a typical example. It shows very distinctly the difference between the lignified fibres (f_1) associated with the phloem, and the non-lignified hypodermal fibres (f_2) which lie between the (f_1) group and the epidermis. Chodat and Balicka-Iwanowska³ have shown that the presence or absence of these hypodermal fibres is a character of considerable systematic importance.

In the closely related genus *Moraea* we find parallels both for the ensiform and bifacial leaves of *Iris*. The Section *Eumoraea* (*Diets*) contains ensiform leaves, such as those of *Moraea Macleanii*, Hort. (Fig. 12, p. 304), and *M. Robinsoniana*, C. Moore et F. Muell. (Fig. 11, p. 304), which recall those of the Irises of the *Apogon* Section, &c. But we also meet with a type of leaf which resembles that of the *Xiphium* Section in being

¹ Goebel, K. (1913), p. 285.

² Hooker, J. D. (1880).

³ Chodat, R., and Balicka-Iwanowska, G. (1892).



FIGS. 36-9. Fig. 36, *Moraea polystachya*, Ker-Gawl.; Fig. 36A, transverse section leaf (x 14); Fig. 36B, transverse section midrib region of another leaf (x 47); Fig. 37, *M. bituminosa*, Ker-Gawl., transverse section leaf (x 14); Fig. 38, *M. sisyrinchium*, Ker-Gawl.; Fig. 38A, leaf apex seen from the ventral side (x 1) to show solid apex, *pet.*, and bifacial region, *sh.*; Fig. 38B, transverse section (incomplete) of bifacial region of leaf (x 14); *m.r.*, midrib region; *m.l.*, main lateral; Fig. 38C, transverse section apical limb of leaf, *pet.*, in Fig. 38A (x 14); Fig. 38D, transverse section midrib region indicated by bracket in Fig. 38B (x 47); Fig. 39, *Salvia verticillata*, L. (Labiatae) transverse section petiole (x 14) to show absence of median bundle.

prevailing dorsiventral, but terminating in a solid monofacial apex.¹ Figs. 37 and 36 show sections of the bifacial leaves of *M. bituminosa*, Ker-Gawl., and *M. polystachya*, Ker-Gawl. Balicka-Iwanowska² states that,

¹ Ross, H. (1892-3).

² Balicka-Iwanowska, G. (1892-3).

in *M. polystachya*, opposite the larger bundles there are smaller bundles inversely orientated. I am unable to confirm this description; I find the bundles to be all normally orientated and arranged in a single series.

The most striking peculiarity of the dorsiventral *Moraeas* is the unusual character of the midrib region. This region, in the species which I have examined, is thinner than the remainder of the leaf, and its bundles are smaller than the rest and somewhat irregularly placed. This feature is not confined to the dorsiventral species. Figs. 12 A and B, p. 304, show the absence of the median bundle in the ensiform *M. Macleanii*; the shape of the leaf of this species makes the lack of midrib even more conspicuous than in the bifacial forms.

The genus *Moraea* differs from *Iris* chiefly in the absence of a perianth-tube and the fact that the stamens generally cohere. It is commonly said that the *Moraeas* are found to the south and the *Irides* to the north of the Equator. But this can only be maintained if the Barbary Nut, *Iris Sisyrinchium*, L., be ranked as a true *Iris*. This plant, which ranges from Portugal and Morocco to North-west India, resembles the *Irides* in possessing a perianth-tube, but in many other points recalls the *Moraeas*. Though Sir Michael Foster¹ placed it unhesitatingly in the genus *Iris*, and both Pax² in Engler's 'Pflanzenfamilien' and Baker³ in the 'Handbook of the Irideae' take the same line, other authorities have long held that it ought to be regarded as a *Moraea*; it was figured as '*Moraea Sisyrinchium*, the European *Moraea*', in Curtis's 'Botanical Magazine' in 1811 (vol. xxxiii, No. 1407). Mr. W. R. Dykes kindly tells me that his long study of the genus *Iris* has convinced him that this plant should be excluded from the genus and transferred to *Moraea*, which it resembles in its corm and in the sheathing leaves of the inflorescence axis, while Ross⁴ and Chodat and Balicka-Iwanowska⁵ have reached the same conclusion from the leaf anatomy, which is the point that specially concerns us here. The leaf of the Barbary Nut (Fig. 38 A) recalls some of the *Moraeas* in consisting of a flat bifacial region (leaf-base) terminating in a cylindrical apex (petiole). The apical appearance of the leaf exactly corresponds, for instance, to that of *Moraea edulis*, Ker-Gawl., as figured in Curtis's 'Botanical Magazine' vol. xvii, No. 613. The internal structure is shown in Figs. 38 B-D, and it will easily be seen that in the anatomy—especially in the peculiarities of the midrib region—there is an exact reproduction of the features of such a species as *Moraea polystachya* (Figs. 36 A and B). The leaf structure thus tends to confirm the attribution to *Moraea*.

¹ Foster, M. (1892).

² Pax, F. (1888).

³ Baker, J. G. (1892).

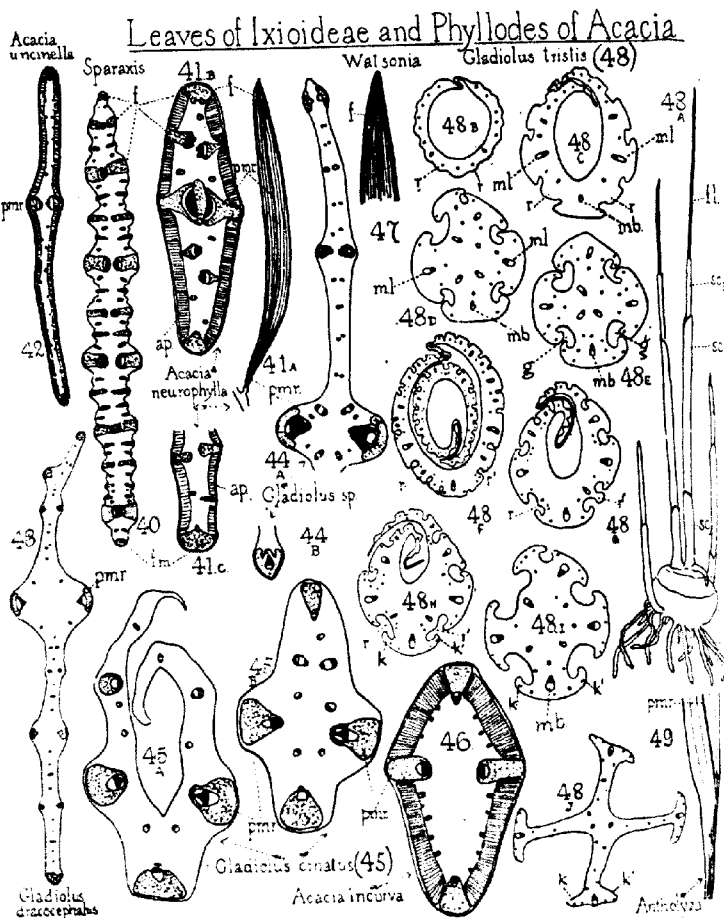
⁴ Ross, H. (1892-3).

⁵ Chodat, R., and Balicka-Iwanowska, G. (1892).

4. LEAVES OF THE IXIOIDEAE AND PHYLLODES OF *ACACIA*.

I showed in the genus *Iris* that the phyllodic leaves range from the vertical ensiform type to the curious 'radial', angular, or ribbed forms of *Iris reticulata*, &c. A corresponding range is characteristic of the Ixioidae. We find a series beginning with typical equitant leaves, such as those of *Sparaxis* (Fig. 40, p. 318), *Antholyza* (Fig. 49), and certain species of *Gladiolus* (Figs. 43 and 44), which can be closely paralleled with the phylloides of such *Acacias* as *A. uncinella*, Benth. (Fig. 42), and *A. neurophylla*, W. V. Fitz. (Fig. 41). The pseudo-midrib¹ (*p.m.r.*), formed from the two main lateral veins, is a striking common character of these Irid and *Acacia* leaves. In addition to the ensiform species, we have also, in the genus *Gladiolus* itself, certain more aberrant forms which may be regarded as developments from the type. *Gladiolus ornatus*, Klatt, for instance (Fig. 45 B), has a leaf in which the ensiform character is becoming modified, and which shows a close resemblance to the phyllode of *Acacia incurva*, Benth. (Fig. 46). *Gladiolus tristis*, L., is a still more remarkable example, which departs to an extreme degree from the usual equitant type; the cruciform section of the limb (Fig. 48 J) does not at first sight suggest a leaf at all. Fig. 48 A shows a plant of *Gladiolus tristis* with a number of young shoots. These each bear a succession of scale leaves (*sc.*₁, *sc.*₂, *sc.*₃) followed by a foliage leaf (*fl.*) indicated in black. The clue to the peculiarities of the leaf structure is to be found in a study of the transition from the basal sheathing region to the limb. Figs. 48 B-E show this transition in the case of the tallest foliage leaf (*fl.*) in Fig. 48 A. At the base the leaf forms a continuous sheath (Fig. 48 B), which is ridged and furrowed, the ridges coming opposite to the bundles. A little higher, the sheath becomes open, and the ridges associated with the midrib and main laterals begin to predominate; the subsidiary ridges (*r.* and *r'*.) have altogether vanished at the higher levels represented in Figs. 48 D and E, their place being taken by the grooves *g.* and *g'*. At the same time the margins of the sheath have fused, and are represented by the fourth main ridge opposite the midrib. Figs. 48 F-J show a series of sections through another leaf, in which the loss of the subsidiary ridges *r.* and *r'* can be followed somewhat more easily than in the preceding series, and in which the ultimate form of the limb (Fig. 48 J) is also included. In connexion with each of the four main ridges a pair of lateral keels or wings (*k.* and *k'* in Figs. 48 H-J) are developed; these wings are supplied by small vascular strands. The outstanding feature of these series of sections through the leaves of *Gladiolus tristis* is that the structure of the basal region of the limb (Figs. 48 D, E, and I) at once suggests that we are dealing with a cylindrical petiolar phyllode, merely modified by four deep invaginations or grooves, associated with slight lateral wings;

¹ 'Côte médiane' of Chodat, R., and Balicka-Iwanowska, G. (1892).



FIGS. 40-9. (Throughout, *p.m.r.*, pseudo-midrib; *f.*, fibres; *f.m.*, fibrous margin.) Fig. 40, *Sparaxis pulcherrima*, Hook. ($\times 14$). Fig. 41, *Acacia neurophylla*, W. V. Fitz.; Fig. 41 A, phyllode ($\times 1$); Fig. 41 B, transverse section phyllode not far from base, two upper bundles not yet united ($\times 14$); *a.p.*, assimilating parenchyma; Fig. 41 C, margin of a similar phyllode (whether dorsal or ventral uncertain) to show great development of fibres in proportion to vascular tissue. Fig. 42, *Acacia uncinella*, Benth., phyllode ($\times 14$). Fig. 43, *Gladiolus dracocephalus*, Hook. ($\times 14$). Fig. 44, *Gladiolus* sp.; Fig. 44 A, adaxial side of limb to show fibrosis of pseudo-midrib and of ventral margin ($\times 14$); Fig. 44 B, dorsal margin of sheath region to show fibrosis $\times 47$; Fig. 45, *Gladiolus ornatus*, Klatt ($\times 47$); Fig. 45 A, sheath; Fig. 45 B, limb. Fig. 46, *Acacia incurva*, Benth., transverse section phyllode ($\times 23$) for comparison with Fig. 45. Fig. 47, apex of leaf of *Watsonia marginata*, Ker-Gawl.; fibrous rim, *f.*, which is bright yellow in herbarium material, indicated in black ($\times 1$). Fig. 48, *Gladiolus tristis*, L.; Fig. 48 A, young plant ($\times 1$); *sc.*, *sc.*, *sc.*, successive scale leaves; *f.l.*, foliage leaf; *c.*, corm; *r.*, roots. (Brown scale leaves have been removed from corm.) Figs. 48 B-E, series of transverse sections from base upwards through tallest leaf in Fig. 48 A, ($\times 23$); *r.*, subsidiary ridges; *g.*, grooves; *m.l.*, main lateral bundles; Figs. 48 F-I, similar series through another leaf ($\times 14$); *A.* and *A'*, keels; *r.* and *r'*, subsidiary ridges. Fig. 49, *Antholyza nervosa*, Thunb., junction of top of sheath and base of limb ($\times 1$).

I think that the comparison with other Irids supports this interpretation. In the upper part of the limb (Fig. 48 J) the invaginations have become so deep that, if we only knew the leaf in this region, its phyllodic character might easily be overlooked.

The sheath of *Gladiolus tristis*, with its ridged and furrowed dorsal surface (Fig. 48 F), is an instance of a type of structure which occurs repeatedly in this family, and to which I shall refer again (p. 332) when considering the general question of the evolution of the Irid leaf.

5. FIBROSIS IN IRIDS AND ACACIAS.

An exaggerated degree of development of the fibrous strands in the leaf is characteristic of many of the Iridaceae. In certain cases the survival of these strands, especially in the basal region, provides a corm-sheath, which is sometimes continued upwards as a tuft of fibres. The most striking instance I have seen is *Antholyza nervosa*, Thunb., in which, in the case of a specimen at the flowering stage in the British Museum herbarium, the fibres are as much as 28 cm. long.

In the individual leaf, the fibrous strands generally run parallel to the bundles, between the phloem and the leaf-surface, but they may also surround and enclose the bundles. In the ensiform leaf-types they are most conspicuously developed at the dorsal and ventral margins. The fibrous rim thus produced—which is rounded in section and often, in herbarium material, bright yellow, polished, and enamel-like—is characteristically confined to the limb of the leaf, and absent from the sheath; it occurs, that is to say, in the member which I interpret as a petiolar phyllode, but not in the leaf-base. *Watsonia marginata*, Ker-Gawl. (Fig. 47), is the most striking instance I have seen; here, both on the dorsal and ventral side, the rim begins abruptly at the junction of sheath and limb. There are also many noticeable cases of marginal fibres in the genus *Gladiolus* (Figs. 43 and 44), e.g. *G. Ludvigii*, Pappe, and *G. crassifolius*, Baker, while various Irises (e.g. *Iris aterna*, L., and *I. Douglasiana*, Herb., Fig. 9, p. 304), *Moraea Robinsoniana*, C. Moore et F. Muell. (Fig. 11, p. 304), *Cypella coriifolia*, Baker, and some Patersonias, show the same characteristic in varying degrees.

From the standpoint of the phyllode theory, the most suggestive feature about the fibrosis of the Irid leaf is the way in which it can be paralleled among the Acacias. The phyllode of *Acacia neurophylla*, W. V. Fitz. (Fig. 41 B), for instance, has a dorsal and ventral fibrous rim, essentially similar to that of a *Gladiolus* or a *Watsonia*. The comparison of Figs. 40, 41, 44, and 47 will show the general agreement of the marginal structure in the two families. The margin of a phyllode seems more liable to fibrous thickening than that of a lamina, possibly because the latter is characteristically attenuated, while the former—not being a true margin.

but representing merely the dorsal or ventral crest of a flattened petiole—naturally tends more to massiveness.

Not only marginal but also apical fibrousness is a common character of Irids and Acacias; the indurated apices of certain 'radial' *Acacia* phyllodes may be compared with the horny leaf-tips of *Iris reticulata*.

It is scarcely possible to cut many sections of the strongly fibrous leaves of Acacias and Irids without being impressed by the apparently excessive character of the fibrosis—excessive, that is to say, from the point of view of any purpose which it may conceivably serve in the plant's economy. The most reasonable view appears to be that the fibrosis is a process outside the plant's control, which may almost be treated as a pathological result of unsuitable conditions. Dr. A. H. Church¹ has recently suggested that the xerophyte is, as it were, embarrassed by excess of waste polysaccharides, and deposits them on its cell-walls simply to get rid of them. The conditions which bring about this excess are, on his view, the disturbance of the balance between photosynthesis and proteid synthesis, due to the inadequacy of the transpiration stream in relation to the available sunlight. The common association of fibres and phloem, and the fact that fibrosis tends to increase towards the leaf apex (e.g. *Crocus vernus*, Fig. 57 B and C, p. 324, and *Hemodactylus tuberosus*, Fig. 29 E, p. 311), may perhaps be regarded as affording some indirect support to Church's view. If his theory be accepted, any utility which may be attributed to the fibres must be regarded as an entirely secondary matter.

6. FOLIATED LEAVES IN THE IRILOIDEAE AND IXIOIDEAE.

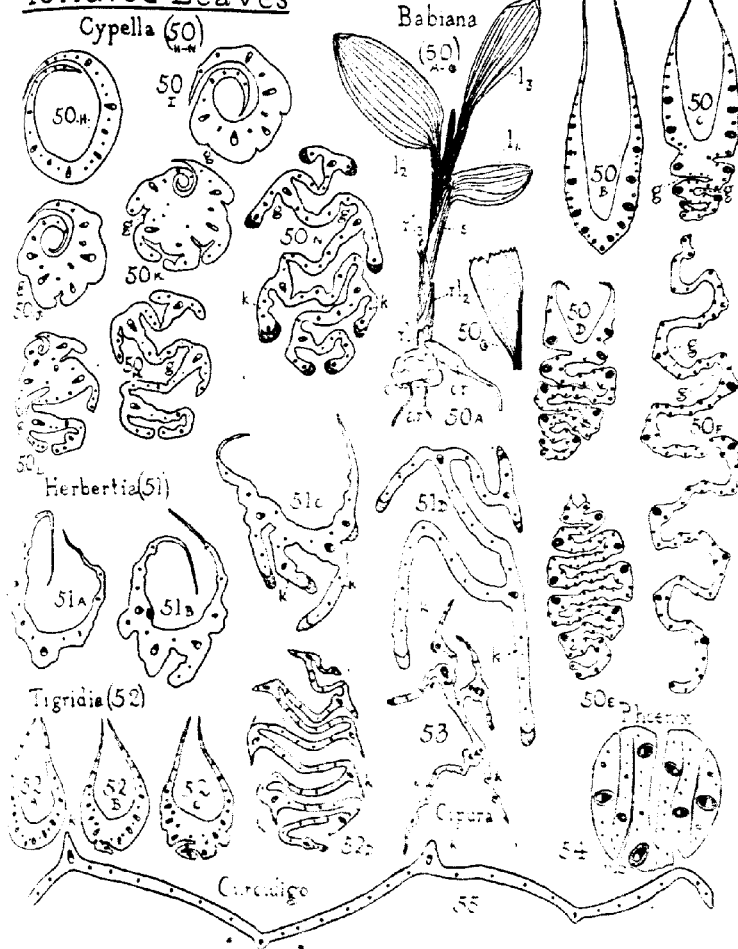
Some of the most aberrant leaf-types met with in the Iridaceae are those for which the names 'folia tabulata' and 'mehrflächige Blätter' have been suggested, and which I propose here to term 'foliated leaves'. As Lindman² rightly points out, these leaves do not differ fundamentally from the ensiform type, though they have been in many cases modified almost out of recognition. The least modified form is perhaps that met with in *Babiana* (Ixioidae). Fig. 50 A shows a typical plant belonging to this genus. The earlier leaves (rd_1 , rd_2 , rd_3) consist chiefly of sheath, with a minute limb in the case of rd_2 and rd_3 . In the typical foliage leaves L_1 , L_2 , and L_3 the limb becomes more and more conspicuous, while the sheath at first sight suggests a petiole. It is by no means rare in Monocotyledons to find that the ancestral loss of the leaf-blade, and the compensatory development of the petiole as a 'pseudo-lamina',³ is associated with the assumption by the sheath of petiole-like functions. Fig. 50 C shows the truncated form of the limb in another species.

¹ Church, A. H. (1919).

² Lindman, C. A. M. (1899).

³ Arber, A. (1915).

Foliated Leaves



Figs. 50-55. Throughout, *g.*, groove; *k.*, keel. Figs. 50A-G, *Babiana*; Fig. 50A, *Babiana* young plant ($\times 4$); *c.*, corin; *a.r.*, absorptive roots; *c.r.*, contractile root; *l.*, *l.p.*, normal leaves; *l.*, leaf sheath; *l.*, *l.*, *l.*, reduced leaves; Figs. 50F-E, series of transverse sections through junction of sheath and limb of *l.*, in Fig. 50A, $\times 14$; Fig. 50G, *Babiana cuneifolia*, Baker, limb and upper part of long leaf-sheath $\times 4$; Figs. 50H-N, *Cypella Herberti*, Herb. $\times 14$; Figs. 50H-N, series of transverse sections through junction of sheath and limb in one leaf; Fig. 50N, limb of another leaf. Figs. 51A-D, *Herbertia pulchella*, Sweet, series through junction of sheath and limb ($\times 23$). Figs. 52A-D, *Tigridia pavonia*, Ker-Gawl., series of transverse sections through junction of sheath and limb ($\times 7$). Fig. 53, *Cypera paludosa*, Aubl., transverse section limb $\times 14$. Orientation uncertain, as only a fragment of the limb was available. Fig. 54, *Phoenix dactylifera*, L., transverse section near base of first foliage leaf ($\times 14$) to show 'folding'. Fig. 55, *Carex* sp., transverse section small part of limb near margin; leaf can be folded up like a fan ($\times 14$).

Babiana cuneifolia, Baker. The leaf-limb in this genus gives, at first glance, the impression of being folded in a fan-like fashion, but this impression is shown to be incorrect when a series of sections is cut through the junction of sheath and limb. Figs. 50 B-F represent such a series for the case of leaf l_3 in Fig. 50 A. Fig. 50 B shows the sheath-region, which is quite normal. In Fig. 50 C the limb is beginning to put in an appearance, but it is modified from the ensiform type by deep grooves or invaginations (*g.*), which occupy alternating positions to right and left. As we leave the sheath region, the grooves become more conspicuously developed (Figs. 50 D and E) until in Fig. 50 F the mature form is reached. These sections show plainly that—as Lindman¹ has already pointed out—the terms 'feuilles plissées'² and 'foglie piegate',³ which have been suggested for these leaves, are inadmissible. We are not dealing with a case of 'folding', but of invagination, which is an entirely different thing. The difference is, in fact, as fundamental as that recognized by the geologist between the case of the production of certain mountain ranges by the actual wrinkling of the earth's crust, and the case of the carving of a system of hills and valleys, by the action of rain and rivers, out of an originally flat surface. For comparison I have included Fig. 54—a section of the basal region of a leaf of *Phoenix dactylifera*, L. (Palmae)—since this leaf has been described as offering a genuine instance of folding. It will be seen from this figure that, if the leaf were flattened out, all the bundles would be normally orientated, with xylem upwards and phloem downwards. And I have also found the same thing to be true of the 'plicated' leaves of *Curculigo* sp. (Amaryllidaceae, Fig. 55) and *Carludovica Plumerii*, Kunth. (Cyclanthaceae). But, on the other hand, if the leaf of *Babiana* be flattened, the bundles will still be orientated in two opposite ways, as in so many other phyllodes. I propose in later papers to consider the nature of the 'folded' leaves of the Palms, Cyclanthaceae, *Curculigo*, &c., and to discuss the relation of these leaves to those of the Irids.

Tigridia (Iridoideae, Figs. 52 A-D) may be taken to represent a further stage in the evolution of the foliated leaf. The transition region between sheath and limb shows that pseudo-plication arises exactly as in *Babiana*, through lateral invaginations. The form is, however, rendered a little more complicated by the development of a slight keel, *k.*, from the leaf surface in the region outside the phloem of each of the main bundles: this keel may itself contain a bundle. Similar keels are also occasionally developed in non-foliated leaves. They may, for instance, occur in connexion with the midrib of an ordinary ensiform leaf, such as that of *Tritonia*,⁴ or with the angles of a tetragonal leaf, such as that of *Hermodactylus* (Fig. 29 B).

¹ Lindman, C. A. M. (1899).

² Chodat, R., and Balicka-Iwanowska, G. (1892).

³ Ross, H. (1892-3).

⁴ Arber, A. (1918), p. 486, and Figs. 15 B and C, p. 483.

p. 311), while—outside the Iridaceae—non-vascular keels are associated with the main bundles of the plicate leaf of *Curculigo* (Fig. 55).

A further stage in the complexity of the foliated leaf is reached in *Cypella* (Figs. 50 H-N), *Herbertia* (*Alophia*¹) (Figs. 51, A-D), and *Cipura* (Fig. 53), all belonging to the Iridoideae. Here the keels (*k.*, *k'*, &c.) are developed into conspicuous lamellae, supplied by a number of bundles, and usually standing out at right angles to the plane of symmetry of each of the main vascular strands. *Cypella Herberti*, Herb., is of special interest in connexion with the phyllode theory, because, just at the junction of sheath and limb, the outline of the transverse section is almost circular (Fig. 50 K). At this stage the leaf is not unlike that of *Gladiolus tristis* at a corresponding level (Fig. 48 D, p. 318). The study of the transition from sheath to limb thus removes any difficulty which might have been felt in visualizing the limbs of both these leaves as cylindrical petioles, which at the extreme base retain some approximation to a typical petiolar structure, but higher up become rapidly modified. The final form to which the limbs attain is markedly different in the two cases (cf. Fig. 50 N, and Fig. 48 J, p. 318). Their ultimate differences depend upon the fact that *Gladiolus tristis* has only four grooves, arranged as two symmetrical pairs, while each main bundle is associated with two lateral wings, whereas *Cypella Herberti* is less symmetrically modified—the grooves, which are more numerous, are placed alternately, and there is only a single wing in connexion with each main bundle.

7. THE LEAVES OF THE CROCOIDEAE.

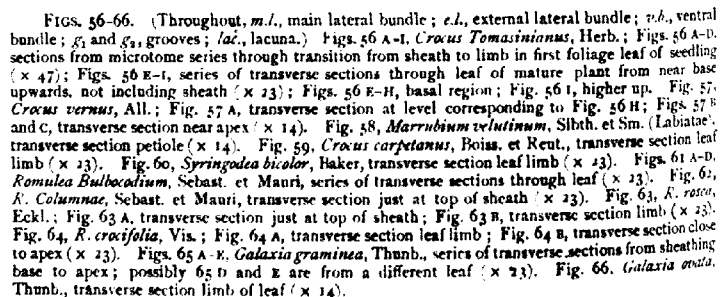
While the Iridoideae contains 35, and the Ixioidae 18 genera, the third tribe of the Iridaceae, the Crocoideae, includes only the four—*Romulea*, *Crocus*, *Syringodea*, and *Galaxia*.² We may first consider the two larger genera, *Romulea* and *Crocus*. Of these *Romulea* has by far the more extensive distribution: it is found in the Mediterranean region and also in Africa (including South Africa), while in the other direction it even extends into England—*R. Columnae*, Sebast. et Mauri, occurring near Dawlish. The genus *Crocus*, on the other hand, is not represented outside the Mediterranean region. The distribution thus indicates that *Romulea* is probably the older type, and certain structural features tend to support this view. For instance, the extreme reduction of the whole *Crocus* plant³ and the length of the flower-tube may be regarded as characters in which the genus has progressed farther than *Romulea* on the path of specialization. The leaf of *Romulea* also diverges less from the average Irid type than does

¹ This case has been studied in detail by Lindman, C. A. M. (1899).

² Pax, M. (1888), and Baker, J. G. (1892). It is possible that *Galaxia* ought to be transferred to the Iridoideae; see p. 318.

³ Church, A. H. (1908).

Crocus Tomasinianus (56) *Crocus vernus* (57) *Crocus Tomasinianus* (56)



that of *Crocus*.¹ So it may be well to begin our consideration of the Crocoideae by trying to make out the nature of the leaf of *Romulea*, and then to consider the relation borne to it by the highly peculiar leaf of *Crocus*.

The leaf structure of *Romulea* is illustrated in Figs. 61-4. *R. Bulbocodium*, Sebast. et Mauri, is a typical example. Here there is a sheath, closed at the base (Fig. 61 A), whose most striking feature is the relative unimportance of the median bundle (*m.b.*) as compared with the main laterals (*ml.*). Higher up, the sheath opens out (Fig. 61 B) and the passage into the limb (Figs. 61 C and D) shows that the leaf is essentially of the ensiform type, not unlike that of *Gladiolus ornatus* (Fig. 45 B, p. 318), but modified by the presence of two pairs of grooves or invaginations, one pair (*g.*) lying between the median bundle and the main laterals, and the other pair (*g.*) between the main laterals and the ventral bundle formed by the fusion of the external lateral bundles (*el.*). *Romulea Columnae*, Sebast. et Mauri (Fig. 62), and *R. rosea*, Eckl. (Fig. 63), do not differ essentially from the type of *R. Bulbocodium*. One member of the genus, however, *R. crocifolia*, Vis. (Figs. 64 A and B), diverges from the *R. Bulbocodium* type in being dorsiventral to the extreme apex of the leaf.¹ I interpret the leaf of this species as a reduced form, equivalent merely to the sheathing leaf-base of the other members of the genus, the petiolar limb having been lost. *R. crocifolia* thus bears the same relation to *R. Bulbocodium* that the *Juno* Irises bear to the Irises with ensiform leaves.

The leaf of *Crocus* is described by Ross² as belonging to the dorsiventral type, and is compared by him to the aberrant *Romulea crocifolia*. Čelakovský³ and Velenovsky⁴ take the same view, while Chodat and Balicka-Iwanowska⁵ expressly state that the leaf of *Crocus* is in no way comparable with that of a normal *Romulea*, and Balicka-Iwanowska,⁶ in her later paper, describes it as 'absolutely isolated among the Irideae'. My study of the anatomy and mode of origin has, however, led me to a conclusion wholly different from that of these five writers: I hope to show that the limb of the *Crocus* leaf is a petiolar phyllode, strictly homologous with the leaf of such a *Romulea* as *R. Bulbocodium*, though it is true that transverse sections of the mature leaves of the two genera (cf. Figs. 56 1 and 61 D) would scarcely suggest the possibility of any relationship between them.

We may take *Crocus Tomasinianus*, Herb., as a case for description. Figs. 56 A-D represent sections from a microtome series through the base

¹ Ross, H. (1892-3), draws attention to the dorsiventral character of *R. crocifolia*, Vis. According to Beguinot, A. (1907-9), this specific name is merely a synonym of *R. nitidus*, Klatt, but judging from a leaf from Visiani's type specimen at Padua, generously sent to me by Professor Beguinot, *R. crocifolia* is more completely dorsiventral in structure than *R. nitidus*, and the leaf anatomy certainly suggests that a separate specific name might usefully be retained.

² Ross, H. (1892-3).

³ Čelakovský, J. (1907).

⁴ Velenovsky, J. (1907).

⁵ Balicka-Iwanowska, G. (1892-3).

⁶ Čelakovský, I. J. (1903).

⁷ Chodat, R., and Balicka-Iwanowska, G. (1892).

of the first plumular leaf of a seedling of this species. Near its attachment to the axis the sheath region is closed. This closed basal region, to which attention was long ago drawn by Irmisch,¹ being extremely short, may easily be overlooked, and it is sometimes, indeed, not typically developed; in serial sections through two shoots of *Crocus speciosus*, Bieb., I found that inextricable confusion was produced by extreme telescoping of the internodes, associated with a fusion of the leaf-bases both with one another and with the axis. The vascular anatomy of the sheath in a plumular leaf of *Crocus Tomasinianus* is shown in Fig. 56 A; the median bundle (*m.b.*) is so slightly differentiated as to be scarcely visible, consisting at this stage of a few procambial cells only. The main laterals, on the other hand, are much larger, and their xylem and phloem can already be distinguished. A second smaller pair of bundles occurs, which we may call the external laterals (*e.l.*). A little higher up two invaginations (*g.*₁) come into existence between the main lateral and external lateral bundles. The further history of these grooves can be better followed in Figs. 56 E-I, which were drawn from a series of hand-sections through the base of a mature leaf of the same species. The invaginations, which are first seen in Fig. 56 F, have developed farther in Figs. 56 G and H, and the lateral wings, thus separated from the main part of the limb, increase greatly in length by intercalary growth, and become coiled. In Fig. 56 I the final stage is reached—the unrolling of the two wings having produced the curious transverse section which characterizes the mature *Crocus* leaf. I have also studied the development of the leaf in *Crocus biflorus*, Mill., *C. speciosus*, Bieb., and *C. vernus*, All.² (Fig. 57), and I find that in each case the history is closely similar to that just traced for *C. Tomasinianus*. In *Crocus vernus* I followed the structure to the leaf-tip and found that at the apex the wings become reduced and the invaginations gradually die out (Figs. 57 B and C), so that there is an eventual return to something approaching the unmodified form of the limb.

When we turn to the comparison of *Crocus* and *Romulea*, we find that the leaves are really built on the same plan, with, however, two differences, which—though non-essential—result in a conspicuously different final form. In both genera we get a pair of grooves or invaginations (*g.*₁) arising between the main laterals (*m.l.*) and the external laterals (*e.l.*), but in *Romulea* there is an additional pair of grooves (*g.*₂) lying between the median bundle and the main laterals; this pair is unrepresented in *Crocus*. Again, in *Romulea* the external laterals (*e.l.*) approach one another and finally fuse to form a single bundle (*v.b.* in Figs. 61 D and 63 B), whereas the process of invagination and winging in *Crocus* carries the two external laterals far

¹ Irmisch, T. 1850.

² Pax, F. (1885), figures the leaf of *Crocus vernus* as possessing no midrib: this, according to my observations, is an error.

apart, so that they are eventually separated by the entire width of the flat leaf. We have already shown that the leaf of *Romulea* is but little modified from the ensiform type—that is to say, it is easily derivable from the most widespread form of petiolar phyllode. If my interpretation of the limb of *Crocus*, as precisely equivalent to that of *Romulea*, be accepted, the *Crocus* leaf must also come into the category of petiolar phyllodes; indeed, apart from the comparison with *Romulea*, the structure of the *Crocus* leaf, considered in itself, carries indications that this point of view is not too far-fetched. Fig. 56 C shows how petiole-like is the stage passed through just above the sheath region of the first plumular leaf in *C. Tomasinianus*. A comparison of Fig. 56 F and Fig. 58, again, reveals how closely the stage at which the invagination is just beginning may be compared with a certain Dicotyledonous petiole, that of *Marrubium velutinum*, Sibth. et Sm.;¹ we even get a corresponding grooving and the same conspicuous pair of main laterals; but whereas the median bundle in *Crocus* is present, though extremely reduced, in *Marrubium* it is altogether absent.

With minor variations, the type of leaf described seems to be universal in the genus *Crocus*, except in the case of two species confined to Spain, *C. carpetanus*, Boiss. et Reut., and *C. nevadensis*, Amo et Campo.² I have not been able to get material of *C. nevadensis*, but I have cut sections of *C. carpetanus*, which has a leaf differing conspicuously from that of a typical *Crocus*. There is a closed sheath at the base, which passes into a somewhat asymmetrical bifacial leaf, traversed by a series of bundles, alternately large and small; the dorsal surface is ridged opposite each of the larger bundles (Fig. 59). The median bundle is not, as in the other *Crocuses*, smaller than the main laterals. This leaf I regard as morphologically a leaf-sheath—the petiolar limb of the other species of *Crocus* being unrepresented. But it is very different even from the sheath region of the other *Crocuses*, and rather recalls such leaves as that of *Moraea bituminosa* (Fig. 37, p. 315). The curious isolation of the leaf anatomy, in association with certain other aberrant features of the plant to which Maw draws attention, arouses some doubt as to whether the inclusion of *C. carpetanus* in the genus *Crocus* can be justified.

The small genus *Syringodea*, which is confined to the Cape, resembles *Crocus* and differs from *Galaxia* and *Romulea*, in having no above-ground stem. Ross³ describes the structure in *S. montana*, Klatt, as dorsiventral, with a convex lower surface and concave above. I have examined *S. bicolor*, Baker, and I find that the structure corresponds in general to Ross's brief description of *S. montana*. There is no strict symmetry about a midrib, and the main laterals (*m.l.*) are larger than the median bundle (*m.b.*), which does not occupy precisely the middle line of the leaf (Fig. 60). The only *Crocus* to which such a leaf could be compared is *C. carpetanus*, and even

¹ Cf. Petit, L. (1887).

² Maw, G. (1886).

³ Ross, H. (1892-3).

here the resemblance is not close. *Syringodea* seems rather to belong to the type of the bifacial species of *Galaxia* and *Moraea*.

Of the genus *Galaxia*, which is also confined to the Cape, I have examined two species. One of these, *G. graminea*, Thunb. (Figs. 65 A-E), shows that lack of symmetry which we have noticed as not unusual among the Iridaceae. It has a sheath (Fig. 65 A) passing into a solid limb (Fig. 65 C), which I interpret as a petiolar phyllode. The anatomy supports this view; the structure, especially in the region near the apex (Figs. 65 D and E), recalls that of the type of ensiform leaf in which the bundles alternate. In another species, *Galaxia ovata*, Thunb. (Fig. 66), the only leaf which I have been able to examine was entirely dorsiventral, but it was probably an early leaf which had not achieved the mature form, since, according to Ross's¹ description, the leaves of this species normally terminate in a short monofacial apex. It is evidently a species in which a typical petiolar phyllode, such as that found in *Galaxia graminea*, has been reduced until it consists chiefly of the leaf-base. The median bundle is unimportant as compared with the main laterals, and the general structure resembles that of various *Moraeas*. It may be recalled that the union of the stamens of *Galaxia* into a tube is also a character in which it approaches *Moraea* and differs from the other Crocoideae. It is quite possible that the genus ought to be removed into the Iridoideae, as has been suggested by Chodat and Balicka-Iwanowska.²

8. ASYMMETRY IN CERTAIN IRID LEAVES.

A tendency to the reduction or loss of the median bundle, sometimes associated with a general lack of foliar symmetry about the adaxial-abaxial plane, is exhibited in many genera of Iridoideae and Crocoideae, though not, so far as I am aware, in the Ixioideae. In the *Xiphium* Section of the genus *Iris*, the midrib is, as a rule, insignificant in comparison with the laterals (e.g. *Iris xiphioides*, Figs. 31 C and D, p. 313). In the genus *Moraea* the midrib region is often thinner than the rest of the leaf, and traversed by several small bundles, of which sometimes no individual can be reckoned as actually median. This peculiarity is not confined to the leaf-sheath leaves (Figs. 36-8, p. 315), for there may be a complete absence of a median bundle in the dorsal region of an ensiform petiolar phyllode belonging to the genus (Figs. 12 A and B, p. 304). According to Ross's³ figures, there is also no median bundle in *Bobartia* and *Ferraria*. In the Crocoideae, *Romulea* (Figs. 61-4, p. 324), *Crocus* (Figs. 56 and 57), and *Syringodea* (Fig. 60) are characterized by median bundles which are smaller than the main laterals, while in *Galaxia* (Figs. 65 and 66, p. 324) and *Syringodea* (Fig. 60) this reduction of the median bundle is associated with a lack

¹ Ross, H. (1892-3).

² Chodat, R., and Balicka-Iwanowska, G. (1892-3).

³ Ross, H. (1892-3).

of general symmetry which makes it scarcely possible to identify a midrib. These conditions can be paralleled in the leaves of some of the Liliaceae,¹ and in certain Dicotyledonous petioles and leaf-bases. For instance, a small median bundle and larger main laterals are found in certain species of *Astelia*, *Allium*, and *Arnocrinum* (Liliaceae), and in the petiole of *Antigonon* (Polygonaceae), while the leaf-base phyllode of *Ancmarrhena* (Liliaceae) and the leaf-sheath of *Foeniculum* (Umbelliferae) resemble the leaf of *Crocus carpatanus* in showing no obvious symmetry about a midrib. I do not know, however, of any cases in the Liliaceae exactly corresponding to that of the Moraceae in which there is no median bundle (e.g. *M. Macleani*, Figs. 12 A and B, p. 304, and *M. polystachya*, Figs. 36 A and B, p. 315), but the petioles of certain Labiates (*Salvia Verbenaca*, Fig. 39, p. 315, and *Marrubium velutinum*,² Fig. 58, p. 324) show a corresponding lack of a median strand.

9. THE EVOLUTIONARY HISTORY OF THE IRID LEAF.

(i) *The primitive character of the ensiform leaf-type.*

In considering the evolution of the Irid leaf, the first necessity is to arrive at some working hypothesis as to what foliar type is to be regarded as primitive for the family. As I have shown on pp. 302 and 303, the typical ensiform (isobilateral equitant) leaf is met with in more than 30 of the 57 genera, including members of both the principal tribes, Iridoideae and Ixioidae. Not only is this form of leaf characteristic of the majority of Irids, but many of the other leaf forms met with in the family—however superficially different—prove on examination (as indicated in the preceding sections of this paper) to be easily derivable from the ensiform type. Among the Liliiflorae, it is not only in the Iridaceae that the ensiform leaf occurs—it is found also in the other two great families, the Liliaceae and Amaryllidaceae. Its distribution, both within the Iridaceae and in the Liliiflorae in general, justifies us in treating it as, in all probability, representing the leaf-type of the original Irid stock. The idea is consistent with Baker's³ suggestion that *Hexcardia* (of the Colchicaceae) forms 'an excellent connecting link between Liliaceae and Iridaceae'—for in this monotypic genus the leaves are ensiform.

Within the genus *Iris* itself, there seems good reason to consider the ensiform leaf-type as primitive. Such a conclusion certainly follows if we accept the deduction from Dr. Willis's 'Law of Age and Area'⁴ that, within a limited group, the more widely ranging types are likely to be the

¹ Arber, A. (1920), pp. 449, 461, and Figs. 1 and 34.

² Petit, L. (1887); in the case of *Salvia Verbenaca* I have failed to find the small median bundle figured by Petit.

³ Baker, J. G. (1880).

⁴ Willis, J. C. (1914).

more primitive. There is no single species of *Iris*¹ covering the range of the genus, but the rhizomatous Section *Apogon*, with its ensiform leaves—to which the widely ranging *I. Pseudacorus*, L., and *I. foetidissima*, L., belong—taken as a whole, covers the entire area of distribution of the genus, from the Pacific Coast of North America in the West, to China and Japan in the East. The members of this Section far outnumber those of the other subdivisions of the genus, and their numerousness, as well as the non-bearded perianth segments, may be taken as indications of the antiquity of the type. Ensiform leaves are also typical of the other large rhizomatous Section, *Pogoniris*, as well as of several smaller Sections of the genus. The bulbous Sections, *Tetragonae*, *Xiphium*, and *Juno*, on the other hand, have a non-ensiform leaf. There seems to be some degree of probability that in the Iridaceae the rhizome may have preceded the bulb. Among the Liliaceae the two related tribes which show the most generalized characters—*Melanthoideae* and *Asphodeloideae*—are prevailingly rhizomatous; it appears likely that it is from some such Liliaceous stock that the Irids have arisen. That the bulbous Irises represent a specialized and relatively late development is also indicated by the fact that, in comparison with the rhizomatous types, they are confined to relatively restricted areas. The *Tetragonae*, whose leaves may, as I have shown, be regarded as a variant upon the ensiform type, are centred about Asia Minor and the Caucasus; the *Xiphiums*, which show transitions towards leaf-base leaves (p. 312), belong to the Iberian and North-west African regions; the *Juno* Irises, whose leaves are reduced to leaf-bases pure and simple (p. 314), though they are found from the Mediterranean to North-west India, cannot compete in range with the ensiform *Apogon* Section.

The Irises are confined to the Northern Hemisphere. That they represent a very ancient stock which has had a long time to differentiate, is indicated by the fact that the genus has a wider area of distribution than any other within the family: another point which also suggests the great age of the *Iris* type is that, with the one exception of *I. setosa*, Pall.,² all the American species are endemic. In Africa, south of the Atlas Mountains, Irises are absent, and their place is taken by the closely related genus *Moraea*, one species of which extends into Australasia. In the *Moraeas* we find typically ensiform leaves resembling those of the majority of Irises, as well as reduced leaves recalling those of the *Xiphium* Section. These two genera—*Iris* and *Moraea*—together range over almost the whole world except South America. The place of the Iridaceae, in tropical and sub-tropical America, seems to be taken by the Maricineae, of which *Marica-*

¹ On the distribution of members of the genus *Iris*, see Dykes, W. R. (1913).

² See Dykes, W. R. (1913), pp. 93, 94. This *Iris*, of which a variety of forms are known to come true from seed, occurs in Northern Asia and passes into America through Kamchatka and Alaska. It is thus possible that it entered America in relatively recent times, though sufficiently long ago to have become differentiated in that continent into a variety of distinct types.

the largest and most widely spread genus—has an ensiform leaf. It is also significant that *Marica* is rhizomatous, whereas the other two genera, *Trimesia* and *Cypella*, are bulbous. The differences between *Iris*, *Moraea*, and *Marica* seem to be of quite subordinate importance, and I think we may reasonably regard these genera as the three modern representatives, differentiated in the Northern Hemisphere, Africa, and South America respectively, from one primaeval world-ranging Irid type,¹ and still retaining many of its generalized characters, including the ensiform leaf.

(ii) *The progression from the ensiform leaf-type.*

Under the ensiform type we must include not only the typical equitant phyllodes, such as those illustrated in Figs. 1-13, p. 304, but also the forms, such as *Sisyrinchium junceum* (Fig. 27, p. 311), which only differ in the matter of proportion from the ensiform leaf of other members of the same genus—the width being greater relatively to the distance between the dorsal and ventral margins. How easy the passage from the truly ensiform to the cylindrical types can be, is seen in the genus *Acacia*, where there are ensiform phyllodes such as *A. pendula* (Fig. 14, p. 304) and *A. uncinella* (Fig. 42, p. 318), and, at the other end of the scale, cylindrical phyllodes such as that of *A. teretifolia* (Fig. 28, p. 311). In the genus *Iris* we get a similar progression from the ensiform types, through the limb of *I. xiphium*, with its oval section (Fig. 32 B, p. 313), to the cylindrical limb of *I. xiphioides* (Fig. 31 D, p. 313).

From the ensiform leaf—using the term in the wide sense—the course of evolution shows signs of having proceeded in two directions, the petiolar limb being either reduced in all degrees down to the point of actual elimination, or else elaborated until it sometimes forms a highly complex pseudo-lamina.

The leaf-bases of equitant leaves in many cases show a marked capacity for development: for instance, in *Micranthus alopecuroides*, Eckl., the leaf-sheaths may be extremely long. In certain genera the leaf-sheath is so far developed as to play the more conspicuous rôle, while the petiolar limb either remains as a well-marked organ or is reduced to a mere vestige. In a previous paper² I have discussed the occurrence of such leaves in the Liliaceae; in the Iridaceae instances are found in the genera *Iris* (*Xiphium* Section) (p. 312), *Moraea* (pp. 314-15), *Hemerocallis*, *Hexaglottis*, and *Galaxia*.³ In the literature, these cases are not referred to in the terms of the phyllode theory which I have here employed, but the leaves are merely described, non-committally, as bifacial with a monofacial apex.

In the *Juno* Irises reduction has gone still farther and the leaves are

¹ For an exposition of the view that all the great families of flowering plants have passed through a period when they existed in an undifferentiated world-ranging form, see Guppy, H. B. 1918.

² Arber, A. 1920.

³ Koss, H. 1892-3.

exclusively of leaf-base nature. In this Section the bristle-like inner perianth segments also suggest reduction-specialization. It should be recognized that the passage from the petiolar phyllode to the leaf-base leaf, though *morphologically* a reduction, may, from the standpoint of *function*, represent an advance. The leaf-bases of the *Juno* Irises, for instance, approach more nearly to true laminae, in structure and orientation, than do the petiolar phyllodes of the rest of the genus. The approximation to a normal Dicotyledonous blade, reached on this line of progression by the loss of the petiole and the exaggeration of the leaf-base, is arrived at on the second line of progression by a converse process—namely, *elaboration* of the petiole; by increase of surface relatively to sectional area, the limb is modified into a pseudo-lamina, which is a more powerful instrument for performing the work of the leaf. This elaboration seems to depend essentially upon invagination or grooving, which may or may not be associated with the production of keels or wings. Grooving of the leaf-tissue between the bundles is highly characteristic of the family; it occurs conspicuously in the leaf-base leaves of *Crocus carpetanus* (Fig. 59, p. 324) and *Moraea bituminosa* (Fig. 37, p. 315), as well as in various petiolar leaves. In the latter the invagination may be so slight as to result merely in a series of ribs and channels, such as those seen in *Iris xiphioides* (Figs. 31 C and D, p. 313) and *Romulea* (Figs. 61-3, p. 324), or it may go much farther, and, as we have seen in the section on Foliated Leaves (pp. 320-3, and Figs. 50-3, p. 321), it may produce the curious pseudo-plicate winged leaves of *Cypella* &c. Even the anomalous leaves of *Crocus* are susceptible, as I have shown on p. 325, of explanation on the same lines. The changes which take place in the petiolar phyllodes of the Irids, in their effort—if we may use the term—to become more effective assimilating organs, recall the peculiar stem developments which arise in the succulent Cactaceae and Euphorbias when the axis takes on the work normally accomplished by the leaf; in both cases we find corresponding morphological changes associated with the assumption, by a radial or approximately radial organ, of work which requires a large surface. The remarkable winged stem of *Euphorbia grandicornis*, which, as Goebel¹ points out, exposes a surface scarcely inferior to that of a leafy shoot, may be broadly compared with the foliated petiolar phyllode of *Cypella* or *Herbertia*, which forms an organ in which the ratio of surface to volume must be greater than in many normal laminae.

The ingeniously diversified leaf-forms of the Iridaceae afford an illustration of the principle which I have termed the Law of Loss.² On the phyllode theory, the leaf of the ancestral Irid, in common with other primaeval Monocotyledons, was of a type in which the lamina had been entirely eliminated, and, on the Law of Loss, this lamina having been once

¹ Goebel, K. (1889).

² Arber, A. (1919).

discarded, could never be regained. A review of the Irid leaves leads us to the conclusion that there has been, as it were, an effort in this family to find some substitute for the lost lamina which would be more efficient than the typical petiolar phyllode as an organ of assimilation. The members of the family have all had the same problem to solve, and the same material—the petiolar phyllode—to work upon; they have apparently discovered no methods by which to deal with the situation, except invagination and the development of keels or wings. But we have only to compare the mature leaves of such divergent types as *Gladiolus tristis* (Fig. 48 J, p. 318), *Crocus* (Fig. 56 I, p. 324), and *Cypella* (Fig. 50 N, p. 321), to see what strikingly different results have been attained by the various modes in which these monotonous methods have been applied.

In surveying the whole range of Irid leaf forms, one can scarcely fail to be struck by the more or less parallel developments from the ensiform type which have recurred within different tribes and genera. In the Iridoideae we meet, in the genus *Iris*, with ensiform and 'radial' phyllodes, and also with leaf-base leaves; with both ensiform and cylindrical phyllodes in each of the genera *Sisyrinchium*, *Trimezia*, and *Bobartia*; with foliated leaves in *Cipura*, *Cypella*, and *Tigridia*. In the Ixiodeae there are many ensiform leaves, such as those of *Schizostylis*; ensiform leaves and also approximately radial phyllodes in *Gladiolus*; foliated leaves in *Babiana*. A corresponding series of 'Parallelbildungen' has been recognized in the different genera of Cactaceae; Ganong¹ enumerates a number of characters which recur repeatedly in different lines within the family. One is reminded also of the 'phyletic drift' recognized by Bower² in the case of the ferns.

10. SUMMARY.

In the present paper a general examination of the leaf structure of the Iridaceae is undertaken, in order to see how far the view can be substantiated that the leaf in this family is a *phyllode*, possessing no lamina but consisting of *petiole and leaf-base*, or *leaf-base alone*. It is found that a study of the transition region between sheath and limb often throws light on the morphology of the leaf.

The ensiform (equitant isobilateral) leaf is first discussed, and the conclusion is reached that the evidence—especially the comparison with modified leaves in the genus *Acacia*—negatives the congenital concrescence theory, and points to the ensiform leaf being a petiolar phyllode (pp. 303-6). The semi-equitant leaves of *Phormium* and *Dianella* (Liliaceae) are held not to be comparable with the ensiform leaves of the Iridaceae, but to have attained their curious form as a secondary modification (pp. 306-7). It is shown that not only are ensiform leaves in many respects similar to *Acacia*

¹ Ganong W. F. (1894)

² Bower, F. O. (1918).

phyllodes, but even the minor detail of the association of ensiform leaves and winged axes, sometimes met with in the Iridaceae and other Monocotyledons, can be paralleled in the genus *Acacia* (pp. 308-10). The nature of the 'radial leaves' of *Hermodactylus*, certain species of *Iris*, &c., is discussed, and it is shown that these leaves are merely variants upon the ensiform type (pp. 310-12), and that they lead on to the leaves of certain *Iris*es and *Moraeas*, which are mainly dorsiventral, but have a monofacial apex; such leaves are regarded as leaf-bases terminating in a more or less vestigial petiole (pp. 312-16). The leaves of the *Juno* *Iris*es are interpreted as being exclusively of leaf-base nature (p. 314). The leaves of the Ixiodeae are next considered, and the origin of the cruciform leaf of *Gladiolus tristis* is traced in detail. It is shown that all such leaves are again mere modifications of the ensiform type, and that the leaves of various species of *Gladiolus* can be paralleled within the genus *Acacia* (pp. 317-19). Attention is drawn to the resemblance which many *Iris*es and *Acacias* bear to one another in their tendency to excessive foliar fibrosis, and the significance of this is considered (pp. 319-20). The peculiar foliated leaf-types met with in *Babiana*, *Cypella*, &c., are examined, and it is shown that they arise from a simple petiolar structure, through invaginations, sometimes associated with the development of keels or wings (pp. 320-3). The leaves of the Crocoideae then come under consideration, and it is demonstrated that the gulf hitherto supposed to separate those of *Crocus* and *Romulea* has no real existence, but that the transition from sheath to limb shows that both leaves are petiolar phyllodes of an essentially similar type and that their divergent mature forms are merely the results of differing types of invagination (pp. 323-8). It is pointed out that the tendency to reduction or loss of the median bundle, which seems inherent in the leaves of the Iridaceae, can be paralleled in certain rare cases among Dicotyledonous petioles (pp. 328-9).

Finally, the evolutionary history of the Irid leaf is discussed, and it is concluded that the ensiform petiolar phyllode is probably primary for the family, and that the various leaf-types met with at the present day are to be interpreted either as reduced from the ensiform type by the more or less complete loss of the petiolar region, or else as elaborated from this petiolar region by invagination and winging (pp. 329-33).

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Studies in the Physiology of Parasitism.

VI. Infection by *Sclerotinia Libertiana*.

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With Plate XIV.

THE earliest investigations on the parasitism of *Sclerotinia Libertiana* are those of de Bary (4), who studied the infection of the host plant from ripe ascospores placed in drops of nutrient solution and in pure water respectively. In the former case the spores germinated and produced a vigorous mycelium which at once penetrated into the living healthy tissue of the plant and killed it. When, however, germination took place in pure water, the germ tubes produced were quite unable to penetrate into the living tissue. De Bary concluded that 'the power of infecting is shown by the power of penetrating the membranes, which are evidently dissolved at the points of penetration. Hence it is very probable that this power depends on the presence of a substance which can dissolve a membrane, a ferment in fact, and that this substance is not formed and discharged in sufficient quantity till the germ tube from the spore is properly nourished and developed.' The hypothesis that parasitic fungi which are not wound parasites are capable of secreting a ferment which softens and dissolves cuticle appears to have been generally accepted by the earlier workers. In his classical investigations on the lily disease caused by *Botrytis*, Marshall Ward (10) expressed the opinion that the germ tube of the fungus dissolved the cuticularized epidermal wall of the host. Busgen (3), while discussing the importance of appressoria in bringing the fungus and host into close contact, assumed that the function of these organs was to serve for the accumulation and penetration of toxic material into the host plant. Voges (14) supposed that the mucilage surrounding the spores of *Fusicladium*, in addition to functioning as an adhesive substance, softened and dissolved the cuticle and thus facilitated the penetration of the germ tube. Miyoshi (11) postulated the existence of chemotropic stimuli and emphasized

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the importance of injury to the cells in the interior of the host, thus giving rise to a production of material which diffuses through the epidermis and exerts a chemotropic influence on the fungal hyphae. In the first investigation of the present series on the physiology of parasitism carried out in this laboratory, Brown (2), working on a powerful extract which he obtained from germ tubes of *Botrytis cinerea*, showed that the extract contained no substance capable of dissolving cuticle. He found that such an extract had no effect on such delicate structures as rose petals as long as the cuticle remained unbroken. This important observation was followed by a careful microscopical study by Blackman and Welsford (1) of the early stages of penetration of the bean leaf by *Botrytis cinerea*. They found penetration of the cuticle effected solely by the mechanical pressure exerted by the germ tube. Later, Dey (6), working on the early stages of infection of French Bean pods by *Colletotrichum Lindemuthianum*, found that the mechanism by which the 'infection hypha' penetrated the host was similar to that employed by *Botrytis cinerea*. In both these microscopical investigations conidia were used for infection experiments. In view of the fact that *Sclerotinia Libertiana* does not, as far as is known, infect its host by means of conidia, and also on account of the general acceptance of the view expressed by de Bary that the hyphae of this fungus possess the property of softening and dissolving cuticle, it seemed of importance to examine microscopically the stages of 'mass infection' by hyphae such as occurs here.

METHODS.

Cultures of the fungus were made on potato-mush agar, prune juice agar, and malt extract agar. The potato-mush agar was prepared according to the method of Brown.¹ The prune juice agar was prepared by adding to a 2.5 per cent. decoction of prune juice an equal volume of 3 per cent. agar solution. The malt extract agar used was made by dissolving 20 gm. extract of malt in a litre of water and adding 20 gm. of agar. The medium was then steamed for half an hour to dissolve the agar, 'tubed', and sterilized.

The fungus grows much more quickly on prune juice agar than on potato-mush, or malt extract, agar. A Petri dish of prune juice agar infected at the centre with the hyphae of the fungus and incubated at 25° C. is covered by a continuous coating of mycelium in about a week. After about ten days aerial branches arise, beginning round the edge of the culture and extending inwards, converting the original sparse growth into a white felted mass. Dense white aggregations of the mycelium soon become apparent round the edges of the culture, and after about a fortnight these turn black, forming the characteristic sclerotia of the fungus.

¹ L. C., p. 319.

On potato-mush agar and malt extract agar growth proceeds somewhat slowly at first, forming a dense felted mass of mycelium in contrast to the active sparse growth on prune juice agar. When an area approximately equal to that of a penny is covered in the centre of the dish, growth appears to be inhibited. About a week later a number of sclerotia are formed along the outer margins of the mycelium, which then extends a short distance farther, when growth is again inhibited and another circle of sclerotia is formed. Thus after about three or four weeks the mycelium reaches the edge of the culture medium and a series of concentric rings of sclerotia are formed coinciding with the successive zones of growth. As many as 40-50 sclerotia may be formed in a single, 4 in., Petri dish. After a period of rest the sclerotia germinate and give rise to apothecia from which ascospores are produced. These, as well as the mycelium of the fungus, are capable of bringing about infection of susceptible plants (12).

Leaves of Scarlet Runner Bean (*Phaseolus coccineus*) and Broad Bean (*Vicia Faba*) were used as material for infection. The leaves were cut from healthy plants, washed in sterile tap-water so as to remove any extraneous material, and were placed in a dry atmosphere for a few minutes. They were then placed on damp blotting-paper in sterile Petri dishes. As was observed by de Bary (4), the fungus mycelium makes very poor growth in pure water, and consequently infections were made in drops of turnip juice. The turnip juice was prepared by steaming $\frac{1}{2}$ lb. of peeled and chopped turnips for one hour. The steamed pieces were placed in a muslin bag and the juice expressed. From this quantity of turnips 130 c.c. of turnip juice were obtained. One part of sterile turnip juice added to nine parts of sterile distilled water was the nutrient medium used. Infections were also made in drops of potato-mush agar placed on the surface of bean leaves. A further method adopted was to place cut leaves in Petri dish cultures of the fungus so that the upper surface of the leaves came directly into contact with the growing ends of the hyphae.

It is difficult to control the hyphal concentration, and hence the stage of infection in an infection drop, when pieces of mycelium are used for inoculation. As a rule, blackened areas are apparent under liquid infection drops after about 30 hours. When this stage was reached it was invariably found that the fungus had already penetrated into the host and was causing disorganization of the tissues. As the present investigation is concerned with the earlier stages of penetration the material was fixed at intervals from 17 hours after infection—usually in Flemming's stronger solution diluted with an equal volume of water. Carnoy's fixing fluid and absolute alcohol containing 25 per cent. by volume of glacial acetic acid were also used and gave very satisfactory results. The material was embedded in paraffin and sections cut 5 m. thick. The sections were stained with gentian violet followed by Sudan III (13). The latter stains

the cuticle very deeply, so that the early stages of penetration can be observed. Sections were also stained by Durand's method (7).

OBSERVATIONS.

The mycelium of the fungus grows vigorously in dilute turnip juice prepared as described above. The hyphae are colourless, rather closely septate, and much branched. In hanging-drop cultures numerous cross-connections are established after 24 hours, and in a few days a dense white web of mycelium is formed. At a later stage the fungus produces numerous structures resembling conidia in grape-like clusters on conidiophores after the manner in which conidia are borne by *Botrytis*, but these structures, whatever their true nature may be, have not so far been induced to germinate.

In hanging-drop cultures of turnip extract the hyphae are enveloped in a thick mucilaginous sheath, which can be easily demonstrated by mounting a piece of actively growing mycelium in Indian ink, as was employed by Errera (8) to demonstrate the gelatinous sheath round the filamentous algae. In such preparations the sheath appears as a clear halo round the hyphae against a black background (Pl. XIV, Fig. 1). After standing for a few minutes the particles of ink became aggregated along the margin of the sheath, giving it a much darker appearance than the general background. The presence of the sheath can also be demonstrated by staining with dilute aqueous gentian violet for 30 seconds and mounting in water. It is broadest round the main branches of the hyphae (Fig. 2) and gradually becomes narrower towards the growing tips (Fig. 1). By means of this mucilaginous sheath the mycelium adheres to the surface of the host and consequently shows little or no tendency to become washed off in the fixing and subsequent operations. In fixed and stained preparations it can no longer be observed as such, but its remains are sometimes visible as fine threads which connect the hyphae to the substratum (Fig. 6).

When the tips of actively growing hyphae in a hanging-drop preparation come into contact with the glass they form short branches on which arise tufts of secondary branches, and thus large appressoria are formed, by means of which the mycelium adheres firmly to the cover-slip. These also are surrounded by a mucilaginous sheath which shows up very distinctly when mounted in Indian ink (Fig. 5).

In four-day-old hanging-drop cultures of the fungus in turnip juice many of the appressoria are observed to show very distinct hyaline circular areas on the surface of the appressorial branches in contact with the glass—giving the impression of pores (Fig. 4). One or several such areas may be developed on each branch. From these, small infection hyphae are sent out, which, in nutrient media, grow into normal vegetative hyphae (Figs. 4 a, 4 b, 4 c). In young appressoria these differentiated areas are not to

be seen although the walls of the individual branches appear to be thickened (Figs. 3, 5). It would thus appear that the contact stimulus induces the formation of a uniformly thick wall round the appressorial hyphae. When further growth is about to take place the thickened walls become locally thinned out, possibly at the first points of contact of the appressorial branches with the cover-slip, and 'infection hyphae' emerge—giving the effect of germ pores when viewed from above. In support of the view that these differentiated areas are secondary formations, and not 'germ pores', it is noticed that when hanging-drop cultures are examined microscopically these areas come into focus before the outline of the appressorial hyphae containing them. This is apparently due to the appressorial branches being pushed downwards by the pressure exerted by the emerging 'infection hyphae' against the cover-slip so that the former come into focus at a lower level. In no case could the hyaline areas and the branches of the appressorium containing them be brought into focus at the same time, as would be the case if they were really germ pores. Further evidence in support of the view that the germ-pore appearance is due to 'infection hyphae' emerging from the appressorium will be alluded to later when dealing with the formation of these organs on the leaf surface. The fact that these hyaline areas are not to be seen in young appressoria may perhaps account for their not having been recorded by previous workers.

PENETRATION.

The first preliminary to penetration is the firm adhesion of the fungus mycelium to the host surface. This appears to be effected by means of the mucilaginous sheath. When the tip of a hypha comes into contact with any resistant material the character of the outer wall undergoes some change. This modified wall extends a short distance behind the point of contact and is easily recognized in fixed and stained preparations by its greater affinity for stains and also by the fact that its refractive index is different from that of the unaltered wall of the hypha. This modification is much more marked in the walls of appressorial hyphae, and before 'infection hyphae' are produced the thickening is continuous over the tips of the branches (Figs. 11, 18).

The appressoria adhere firmly to the cuticle by means of the mucilaginous sheath. When penetration is about to take place the thickened walls of the appressorial hyphae become locally thinned out over a small area, and in longitudinal section a bright halo is noticed inside the thin portion of the wall as though the latter were being dissolved (Fig. 12). The modified wall over this area disappears and the small infection hypha presses firmly against the cuticle, which may become markedly indented (Figs. 10, 11, 12, 14). Although carefully looked for, it has not been observed that the staining reactions of the cuticle or subcuticular layers

afford any evidence of their being softened or dissolved at this stage, as was described by de Bary (4).

The cuticle appears to be ruptured by purely mechanical means.

When infection takes place without the formation of a large appressorium the essential feature of penetration, viz. the mechanical rupturing of the cuticle, is the same. The pressure exerted on the surface of the host, apparently rendered possible by the mucilaginous investment surrounding the tips of the hyphae, may be very considerable (Fig. 9). As in the case of appressorial penetration an 'infection hypha' is sent out which pierces the cuticle (Figs. 13, 15).

When the barrier offered by the cuticle is overcome the 'infection hypha' swells into a vesicle in the epidermal cell (Figs. 12, 13, 14, 15, 16). Immediately the continuity of the cuticle is broken the walls of the host cells near the point of penetration show signs of being chemically altered. The vesicle is surrounded by a bright halo probably indicating solution of the cell contents (Figs. 13, 15). In some instances the subcuticular layer of the epidermal wall is markedly swollen (Figs. 6, 15), and in extreme cases the swelling of the cellulose walls may completely obliterate the cavity of the epidermal cell (Fig. 15). The swelling of the subcuticular layer appears to be an irregular feature, as no such swelling could be detected in several instances after penetration had taken place (Figs. 12, 14). In such cases, however, in addition to the alteration in the staining capacity of the layer, its distinctly laminate structure indicated that the wall had undergone some chemical change. While the vesicle is still confined to the epidermal cell the chloroplasts of the palisade cells underneath appear to lose their coherence (Fig. 15). They swell up and become converted into a darkly staining uniformly granular mass. Meanwhile the nuclei of the palisade cells beneath the point of attack move towards the top of the cells, as was observed by Blackman and Welsford in the case of infection by *Botrytis cinerea*. Later, they also lose their identity, and are indistinguishable in the general disorganized mass of cell contents. In hand-cut sections of fresh material the affected areas appear as brownish or blackish patches extending for some distance on either side of the point of attack, indicating that death of the cells has taken place. In fixed and stained preparations the affected areas can be easily recognized owing to their increased capacity for absorbing stains.

Death of the cells extends some distance beyond the limits of the invading hypha, due either to enzymes secreted by the fungus or to the products of disorganized cells. The lethal substance or substances appear to diffuse more rapidly along the palisade cells of the mesophyll than into the spongy parenchyma, as the chloroplasts of the palisade cells for some distance on either side of the point of infection are swollen or disorganized, while those of the spongy parenchyma immediately underneath are still

unaffected. This is evidently due to the fact that the palisade cells are in closer contact and hence allow a more rapid diffusion of the lethal substance or substances than the cells of the spongy parenchyma, which are in contact at comparatively few points. The vesicle increases in size and eventually sends branches along the epidermal wall of the host. Meanwhile the original small breach is widened by the contraction of the cuticle, rendered possible by the collapse of the underlying cells, and the ruptured ends are pushed inwards by the continued growth of the fungus (Figs. 16, 17, 18, 19). Very rarely were the hyphae observed to grow downwards into the mesophyll cells of the leaf immediately after penetration.

Even when the fungus has been growing in the epidermal layer for some time and the tissues of the leaf are completely disorganized, the staining reactions of the cuticle indicate that it has undergone no chemical change (Figs. 17, 18, 19).

As was observed by Blackman and Welsford, and also by Dey, the number of infections increases rapidly after the cuticle is once broken. This may be due to a chemotropic stimulus or to the fact that most of the hyphae in the infection drop are about the same stage of development, and hence it would be expected that many would penetrate about the same time. The stomatal apertures do not appear to have any special attraction for the fungal hyphae, as only in one case was the fungus observed to enter by this channel, and in that particular case an appressorium was formed over a stoma, one of the appressorial branches entering through the stomatal aperture while the others penetrated in the normal manner (Fig. 20).

APPRESSORIA.

It has already been noted that Busgen emphasized the importance of appressoria in bringing the fungus and host into close contact. His assumption, however, that these organs serve for the accumulation and penetration of toxic substances into the host can no longer be accepted. More recently Hasselbring (9), working on the formation of appressoria by the anthracoses, found that in that group the food factor was an important one. When grown in nutrient media he observed that the germ tubes lost their power of reacting to contact stimuli by the formation of appressoria. In this connexion the views of de Bary (2), working on the infection of bean plants by *Sclerotinia Libertiana*, are worthy of note. Since the attachment organs formed by this fungus vary considerably in size, it is possible that different workers may interpret these organs differently. It will therefore add to a clear understanding of the experiments to be described to quote de Bary's description of appressoria: ' . . . the mycelium forms short branches on which arise tufts of secondary branches which, becoming

¹ (4) l. c., p. 21.

closely clustered together, are divided by numerous transverse walls into short segments with membranes that become dark brown with time.'

De Bary so arranged one of his infection experiments that the actively growing mycelium of a culture of *Sclerotinia Libertiana* came into contact with the stem of the bean plant after a short passage through damp air. He noticed that under these conditions the fungus did not penetrate into the host directly, but proceeded to form appressoria (Haftbüschel), and while these were being formed he observed that the underlying cells collapsed and became blackened before penetration of the cuticle took place: '... beginnt das Absterben der vom Büschel berührten Zellen bevor der Pilz in und durch die Epidermis ... gedrungen ist.'¹ When the fungus was applied to the stem in a nutrient fluid he observed that penetration took place without the formation of attachment organs. Believing, as de Bary did, in the softening and dissolution of the cuticle, he concluded that the omission of the formation of attachment organs in the later case was due to the fact that the mycelium in the nutrient liquid was better nourished than that which had grown a short distance through the air and consequently secreted the necessary toxin for the softening and dissolving of the cuticle more rapidly, so that the necessary stimulus for the formation of attachment organs was not forthcoming.

Similar conditions of infection were obtained in the present investigation by placing freshly cut bean leaves on the surface of an actively growing culture of the fungus so that the hyphal tips came into contact with the host surface after a short passage through the air. Another method adopted was to infect drops of potato-mush agar on the surface of the bean leaf with the mycelium of the fungus as already described. In the latter case the fungus grew vigorously in the nutrient agar, but was not observed to penetrate the cuticle underneath the drop. Aerial branches were sent out, and when these came into contact with the host surface through the air, penetration was observed to take place. In neither of these experiments were the observations of de Bary on the formation of attachment organs confirmed. Although appressoria are very abundantly formed under conditions of aerial infection (Figs. 10, 11, 12, 14, 18), several instances of penetration by single hyphae without the formation of the characteristic attachment organs were also observed (Figs. 8, 9, 13, 15). It is difficult, therefore, to accept the views of de Bary and Hasselbring that the nutrition of the fungal hyphae controls the reaction to contact stimulus.

As noted by de Bary, penetration from characteristic appressoria has not been observed when infections are made in nutrient liquid on the surface of the leaf. If the absence of appressoria be found to be a constant feature in the case of infection from liquid nutrient drops, the difference in behaviour compared with aerial infection may possibly be due to differences

¹ (5) l. c., p. 413.

in the amount of mucilage present in both cases. It has already been noted that both hyphae and appressoria are surrounded by a thick mucilaginous investment when growing in a nutrient fluid. The mucilaginous sheath is much less constant and more difficult to demonstrate in the case of aerial hyphae. When such hyphae come into contact with the host the pressure exerted by the infection tube may dislocate the hyphal tip owing to the lack of mucilage. This may give rise to the necessary stimulus for the formation of attachment organs.

Whatever may be the true explanation of the difference in behaviour of the fungus in attacking through a liquid medium and through the medium of air, it cannot be explained by assuming that in one case the hyphae are sufficiently well nourished to secrete the necessary toxin to dissolve the cuticle, since penetration can and does take place both by direct infection and by penetration after the formation of attachment organs under similar conditions of food-supply. As already stated, no evidence could be adduced in support of de Bary's observation that death of the underlying cells took place before the cuticle was penetrated.

SUMMARY.

The early stages of infection of bean leaves by *Sclerotinia Libertiana* have been studied.

The hyphae of the ordinary mycelium and also the appressoria growing in turnip juice are surrounded by a mucilaginous sheath. In the case of aerial hyphae the mucilaginous sheath cannot always be demonstrated.

When a hyphal tip comes in contact with any resistant material, such as a cover-slip or the host surface, the staining reaction of the wall of the tip becomes modified. This modification extends a short distance behind the point of contact: it is very strongly marked in the case of appressoria.

From the tip of each hypha which is in contact with the host plant or with a glass surface there arises an 'infection hypha', usually very narrow, which, under appropriate conditions, penetrates the host.

The 'infection hypha' shows a normal unmodified wall.

The cuticle may be markedly indented at the point of contact with the fungal hypha. This indentation is due to the pressure exerted by the 'infection hypha'.

The invading hyphae are apparently fixed to the cuticle by means of the mucilaginous sheath.

There is no evidence at this stage of the softening or solution or any modification of the cuticle or subcuticular layers of the host.

The rupture of the cuticle by the 'infection hypha' appears to be due solely to mechanical action.

After the cuticle has been penetrated the tissue beneath rapidly

becomes disorganized: this disorganization extends for some little distance in advance of the infection hypha.

Under similar conditions the penetration of the host may take place with or without the formation of appressoria.

The method of hyphal 'mass infection' displayed by *Sclerotinia Libertiana* is thus physiologically similar to that of infection by *Botrytis cinerea* and *Colletotrichum Lindemuthianum*.

It is with great pleasure that the author expresses his indebtedness to Prof. V. H. Blackman, at whose instigation the work was undertaken and from whom he has received most helpful suggestions and criticism.

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EXPLANATION OF FIGURES IN PLATE XIV.

Illustrating Mr. Boyle's paper on Infection by *Sclerotinia Libertiana*.

All figures were drawn with the camera lucida. Figs. 1-5 (except Fig. 4) were drawn from fresh material. Fig. 4 was drawn from material fixed and stained in picro-nigrosin.

Figs. 6-7. Host tissue was *Phaseolus coccineus* infected in turnip juice.

Figs. 12, 14, 20. Host tissue *Vicia Faba* infected by laying leaf on pure culture of the fungus.

In remaining figures host tissue was *Vicia Faba* infected from potato-mush agar drop on leaf surface.

Fig. 1. Young hyphae showing mucilaginous sheath: drawn from fresh material mounted in Indian ink. $\times 616$.

Fig. 2. Older hyphae showing thick mucilaginous sheath. $\times 290$.

Fig. 3. Commencement of appressorium formation. $\times 616$.

Fig. 4. Older appressorium fixed and stained in picro-nigrosin: the points of origin of 'infection hyphae' in appressorial branches are very distinct. $\times 616$.

Figs. 4 a-4 c. Stages in the production of an 'infection hypha' from a branch of an appressorium. $\times 773$.

Fig. 5. Young appressorium showing mucilaginous sheath: drawn from fresh material mounted in Indian ink. $\times 832$.

Fig. 6. Young hyphae fixed to surface of leaf by means of mucilaginous sheath: mucilage in one case represented by fine threads. $\times 773$.

Fig. 7. Hypha growing along surface of leaf. $\times 773$.

Fig. 8. Cuticle depressed at points of contact of fungal hyphae. Epidermal cell collapsed owing to presence of fungus in tissues: chloroplasts of palisade cells underneath disorganized: nucleus seen at top of palisade cell. $\times 773$.

Fig. 9. Cuticle further indented. No chemical action apparent. $\times 1,091$.

Fig. 10. Appressorium in contact with cuticle: depression very marked. $\times 773$.

Fig. 11. Large appressorium adhering to cuticle: cuticle shows no signs of being chemically affected: fungus has penetrated elsewhere: lumen of epidermal cell has disappeared and walls are much swollen. $\times 1,091$.

Fig. 12. Penetration has taken place from one of the appressorial branches: the other is sending out an 'infection hypha'. The modified wall is thinned out at the apex: a clear area can be seen in the wall. $\times 1,520$.

Fig. 13. The 'infection hypha' has penetrated the cuticle and is forming a vesicle in the epidermal cell: vesicle surrounded by a clear halo. $\times 1,091$.

Fig. 14. Penetration from an appressorium, with the formation of vesicles in the epidermal cells: modified wall of appressorium very distinct. $\times 773$.

Fig. 15. The 'infection hypha' has penetrated the cuticle and is forming a vesicle: contents of epidermal cells being dissolved: vesicle surrounded by a clear halo: chloroplasts losing their definite outline: nuclei of palisade cells near the top. $\times 773$.

Fig. 16. Penetration from part of an appressorium: vesicle has increased in size: ruptured cuticle being turned inwards by growth of fungus. $\times 1,091$.

Figs. 17, 18. Penetration from an appressorial branch. Fungus is growing along the epidermal layer: ruptured ends of cuticle turned inwards by invading hyphae. $\times 1,520$.

Fig. 19. Hypha growing along epidermis from vesicle: ruptured ends of cuticle pushed inwards. $\times 1,091$.

Fig. 20. An 'infection hypha' has entered through a stoma. $\times 773$.

On Certain Plastids, with Special Reference to the Protein Bodies of *Zea*, *Ricinus*, and *Conopholis*.

BY

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With Plate XV.

IN a recent volume of this journal (vol. xxiii, p. 91) the writer showed that leucoplasts and chloroplasts in certain plants were developed from small granular or rod-shaped primordia present in meristematic tissues, such primordia having been frequently referred to in the literature as mitochondria, chondrioconts, chondriosomes, &c. He maintained that chloroplasts and leucoplasts were morphologically alike; that their primordia were permanent organs of the cell. He endeavoured to show also that in the same cells, along with the primordia of leucoplasts and chloroplasts, other similar bodies were present which, in very young cells, could not be distinguished from the primordia of the above-named plastids, and which did not develop into these plastids. Merely for the sake of clearness and brevity these bodies were referred to as chondriosomes. In certain Liverworts, as *Anthoceros*, *Marchantia*, &c., similar bodies were described and figured which existed in the cells along with the chloroplasts, as had been pointed out by other observers. The term chondriosome was applied to these bodies for the same reason. No function was definitely ascribed to these bodies, but it was suggested that they were probably concerned in certain processes of metabolism (Mottier, 1918, p. 112). With this thought in mind, a study has been made of the development of protein granules in *Zea Mays* and *Ricinus communis*, and of certain bodies giving a protein reaction with the usual microchemical tests in the phanerogamic parasite *Conopholis americana*, (L. f.) Wallr.

The technique used in this study was the same as published in detail in my former paper. It may be added that the post-chromising part of the process was found to be unnecessary.

[Annals of Botany, Vol. XXXV, No. CXXXIX, July, 1921.]

A a

ZEA MAYS L.

For study a variety of sweet corn known as Golden Bantam, and an unnamed variety of starchy dent corn were selected.

The endosperm of *Zea* develops from the periphery towards the centre, but the meristematic region is at the periphery; consequently the younger cells are not next to the embryo. Soon after the cavity of the embryo-sac has become filled with endosperm, and the embryo has reached a considerable size, the several parts having been differentiated, the outermost layer of the endosperm, the aleurone layer of the mature grain, is differentiated as a definite and sharply defined row of cells, isodimensional or somewhat elongated (Pl. XV, Fig. 1). These cells show occasional periclinal divisions, but the more active meristematic cells, especially at a little later stage, lie just beneath, as seen in Fig. 2. With ordinary magnifications these cells present a dense granular appearance not unlike typical meristematic cells. A careful study shows, however, that in the groundwork of cytoplasm numerous very small and densely staining granules and short rods are present, along with others that are larger and globular (Fig. 1). The larger granules are not so numerous at this stage, and at an earlier stage they are not present, or, if present, not readily recognized.

At a later stage the aleurone layer becomes more conspicuous, the rounded granules in the cells, among which are numerous very small granules and short rods, becoming larger and much more numerous. The outer wall of these cells begins to thicken at this stage (Fig. 2). Beneath the aleurone layer is a meristematic region about three cells in depth, in which the elements are flattened in a tangential direction, owing to rapid division in that plane (Fig. 2). In the first row of cells beneath the aleurone layer, protein granules are present, though fewer in number, together with the small rods and granules already mentioned. In the second and third layers, few of the larger, round granules are present at this stage, but the very small rods and granules are quite in evidence. Deeper in the endosperm the cells are large and relatively poor in contents (Fig. 5). They contain large vacuoles and many starch grains, especially in starchy corn. Figs. 2 and 5 were drawn from starchy corn. In some of the cells the nuclei do not present the smooth, even outline, but rather an irregular contour (Fig. 5). In the cytoplasmic groundwork, among the starch grains and in the cytoplasmic strands between the vacuoles, are numerous densely staining granules and short rods similar to those in the aleurone layer and in the meristematic region (Figs. 2 and 5).

With further growth of the grain of corn, the cells of the aleurone layer rapidly increase in size, the walls becoming very thick, especially the outer and radial walls (Fig. 3). The aleurone granules have also increased greatly

in size, the diameter of the larger equalling one-third that of the nucleus. They are very numerous, and, unless the sections are very thin, they give the cells a dense black colour. In a section of an entire grain of corn at this stage of development, the aleurone layer stands out in striking contrast with the rest of the endosperm, although the cells which make up the bulk of the rest of the endosperm are rich in small, globular protein granules which behave towards stains precisely like those in the outermost, or aleurone layer (Fig. 4). Fig. 3 was drawn from the very thin section. As will be seen from this figure the aleurone granules vary in size, and among them are to be observed many very small granules. It is inferred that the larger granules have developed from the smaller. I was not able to convince myself that the very small granules fuse at this stage to make the larger granules. Fusion of the granules may possibly occur at a later stage.

As stated in the foregoing, the protein granules are not confined exclusively to the so-called aleurone layer. Fig. 4 represents three cells immediately below Fig. 3, less highly magnified. The protein granules are much smaller, more uniform in size, and very numerous. They are about as large as the aleurone granules in the aleurone layer at the stage of Fig. 2.

In the first layer of cells beneath the aleurone layer usually no starch is present, although an occasional grain is not unlikely; in the second layer of cells a few starch grains are present, while in the third layer, in which the cells have greatly increased in size, much starch is present (Fig. 4). Figs. 3 and 4 were taken from Golden Bantam sweet corn. In this variety compound starch grains predominate, while in starchy corn the simple starch grains prevail (Fig. 5). The writer is not prepared to say whether this statement is true for all sweet and starchy corn respectively. As we go deeper in the endosperm the globular granules seem to become less numerous, while the starch increases in quantity. In Golden Bantam sweet corn the more deeply lying endosperm cells contain much more starch than the lower cell of Fig. 4, and they are larger. Many of the starch grains, as stated before, are compound, varying greatly in size, and composed of from two to several smaller grains. Among these lie numerous smaller, simple grains. Many of these are just large enough to show a starch inclusion. It is possible, therefore, that some of the smaller granules in the lower cell of Fig. 4 are the primordia of leucoplasts.

When the grain of corn is only a little more mature than the stage from which Figs. 3 and 4 were taken, the aleurone layer and the remaining endosperm become so hard on dehydration that the preparation of thin sections from embedded material is almost impossible. The writer was not so much interested in the further behaviour of these bodies during the final steps in the maturing of the grain of corn as in the origin of the bodies in question.

From the foregoing it is clear to the writer that aleurone or protein granules in *Zea* arise from definite primordia not unlike the primordia of leucoplasts and chloroplasts as regards their morphological and microchemical properties. That is, they fix and stain like the primordia of leucoplasts and chloroplasts.

RICINUS.

The seed of *Ricinus communis* has been a favourite object for a study of the aleurone content of the cell. The earlier observers devoted their studies largely to the morphological structure and microchemical reaction of the mature granules, and the literature on the subject is somewhat extensive. It is not the writer's intention to go into that phase of the subject, as a study of the fully developed aleurone granules can throw but little light upon the precise manner of their origin.

Among those who have given some account of the origin of the protein granules in *Ricinus* are Pfeffer (1872) and Wakker (1888).

According to Pfeffer (1872, pp. 516-87), the protein crystalloids and globoids appear in the earlier phases of their development as soon as the seed-coat has become reddish brown in colour. The cell contents become turbid by the presence of oil-drops and albuminous matter. This granular material is distributed uniformly in the cell-sap, or accumulated about the nucleus and in the plasmic strands. Immediately following this condition small starch grains are to be seen in the cell-sap, which, however, soon disappear. Pfeffer refers to this starch as transitory starch. With the disappearance of the starch other bodies are seen which have become more accessible to observation, because of the disappearance of the starch and because of an increase in size. There are now to be recognized spherical structures, the globoids and the sharp angular crystalloids. As the latter increase in size, they are seen to agree in form with the crystalloid of the ripe seed. During the ripening* of the seed the gradually enlarging globoid and crystalloid lie near each other or in contact in the turbid cell-sap rich in fat. Pfeffer does not, however, trace the formation of the crystalloid to a definite organic primordium, but, according to his explanation, the globoid of double phosphate of lime and magnesia first appears, which then becomes a centre for the formation of protein material. 'Das Nebeneinanderliegen der Krystalloide und Globoide findet vielleicht dadurch seine Erklärung, dass mit der Bildung von Phosphorsäure an Kalk und Magnesia die Ausscheidung derjenigen Proteinstoffe veranlasst wird, welche zum Wachsthum der Krystalloide verwandt werden.'

Both globoid and crystalloid, according to Pfeffer, attain full size about the time the funiculus has begun to dry. Up to this time he did not observe the vacuole-like space in which these structures usually lie, and which he called the 'Hüllmasse', formed by the withdrawal of water in the

ripening of the seed. 'Dass Wasserentziehung die Hüllmasse veranlasst, zeigen evident noch nicht völlig gereifte Samen, welche man nach Entfernung der Schale einige Stunden an der Luft austrocknen lässt. Denn nun findet man eine Hüllmasse, welche in dem Samen zuvor nicht vorhanden war, gebildet.' Pfeffer seems to have had the idea that the protein granules were crystalline in shape from the start, as he states that (l.c., p. 458) Nägeli's explanation, namely, that globular protein granules gradually took the form of crystals, did not hold for *Ricinus*.

Sixteen years after the appearance of Pfeffer's paper, Wakker (1888) published his results of an extended study upon the contents of plant-cells. His account of the formation of protein granules in *Ricinus* is briefly as follows: The formation of endosperm (1888, p. 453) proceeds from the periphery towards the centre. In a nearly mature seed all stages in the development of endosperm cells are to be found. The youngest cells, which lie in the centre of the young seed, have a wall-layer of cytoplasm with a distinct nucleus and a large central vacuole. The cytoplasm is unevenly finely granular. The cells are in active division, and inclusions are lacking. Farther towards the periphery the cells are smaller; the nucleus lies in the centre of the cell and is in connexion with the parietal cytoplasm by numerous fine strands. The granules are numerous and larger, covering the nucleus, and thereby obscuring its sharp outline. Proceeding towards the periphery the plasmic strands of the cells become more numerous, dividing the original vacuole into more or less spherical vacuoles which impart an almost frothy appearance to the cytoplasm. It is in these spherical vacuoles that small bodies appear, lying exactly in the centre and frequently showing molecular motion. In older cells these bodies are larger, and their true nature is seen. They show sharp corners and edges, and we may assume that they are the youngest stages of the crystalloids. The application of reagents demonstrates this with a certainty.

Wakker makes no mention of the presence of starch in the young cells, as pointed out by Pfeffer and by Maschke¹ (1859) and Gris¹ (1864).

Werminski (1888) claims that aleurone granules arise as a precipitation from albumin in solution in vacuoles during the ripening of the seed.

According to the findings of Rendle (1888, p. 162) the aleurone grains of *Lupinus digitatus* first appear as small bodies lying in the cytoplasm. They stain more deeply than the protoplasm itself with iodine, haematoxylin, Hoffmann's blue and eosine, and the staining is perfectly homogeneous. He shows that there is no crystalline or globoid contents in the protein granule, i.e. no solid mineral content, a claim made apparently by Pfeffer. As to the origin of aleurone grains, Lüdtké (1890) agrees with Pfeffer.

De Vries, basing his conclusion upon the investigations of Wakker,

¹ Mentioned by Pfeffer. I have not had access to the original papers of these authors.

makes the sweeping statement that aleurone grains are dried out vacuoles in the seed. 'During the process of ripening (1910, p. 155) the amount of protein matter dissolved in the cell-sap gradually increases until the fluid becomes of a thick, slimy consistency. In drying, some of the protein bodies crystallize and form the well-known crystalloids, while the remaining protein hardens into an amorphous mass around them.'

De Vries, like the majority of his predecessors, regarded protein granules as a separation from the cell-sap as a sort of mother fluid. We are not told how the aleurone came to be in the cell-sap or cytoplasm in the beginning.

As stated by Pfeffer and Wakker, aleurone granules appear in the endosperm of *Ricinus* as the seed approaches its mature size, and when the seed-coat begins to show a brown colour. A cross-section of the seed at this stage, or even a little earlier, shows several stages in the formation of the protein bodies. The cells nearer the developing embryo, that is, near the central part of the endosperm, and those at the periphery, contain no protein granules as these are usually understood. Fig. 6 is intended to represent one of these cells, which is taken from the periphery of the endosperm. Those near the centre show a similar structure. It should be borne in mind that cells immediately bordering the cavity in which the embryo lies may be undergoing dissolution. Such cells show frequently one or a few large vacuoles. At the stage of development shown in Fig. 6, the nucleus is centrally placed in a mass of cytoplasm. About this are vacuoles varying in size. The vacuoles at this stage contain only cell-sap. In the cytoplasm are clearly differentiated many densely staining granules and short rods, along with a number of rounded bodies that are larger, and which often do not remain stained so densely with the degree of differential staining represented by this figure. The larger rounded bodies are doubtless the primordia of leucoplasts, while the remainder are the primordia of other plastids.

Freehand sections of fresh or alcoholic endosperm at this stage treated with iodine in potassium iodide reveal the presence of small starch grains, especially at the periphery and at the inner region of the endosperm near the embryo. These starch grains are small, of variable size, and the blue coloration is not very marked. This represents the transitory starch mentioned by Pfeffer, but it does not appear sufficiently abundant to obscure observation.

In Fig. 11 are shown leucoplasts with their inclusions, taken from the peripheral cells of the endosperm, as they appear in the permanent preparation made according to the technique employed. They are very small and are similar to the leucoplasts found in tissues of other plants, such as *Zea* and *Pisum*. Some are rounded, while others are in the form of a hand-mirror.

A little deeper in the endosperm the contents of the cells become very much denser, and the vacuoles, as shown in Fig. 6, are replaced by more numerous and smaller vacuole-like cavities in which are numerous small bodies varying in size, which stain deeply with the iron-haematoxylin and appear as dense, black, rounded granules or short rods. They are not aggregated in the centre of the cavity at first, as claimed by Wakker, but they are distributed about the periphery and throughout the centre. These bodies seem to be the same as the rods and granules of Fig. 6, save that they are larger, and the impression is that they have collected from the groundwork of the cytoplasm into the vacuole-like cavities. Many of the cavities have a membrane or boundary that appears as a sharp line, heavier than the nuclear membrane, especially at a later stage of development (Fig. 7). In Fig. 8, a slightly older stage, the vacuole-like cavities are a little larger, and the contained granules are also larger. The cavities vary in size, and in younger cells the granules are smaller and, in all cases, numerous (Figs. 7 *b*, 7 *c*). Many of the endosperm cells are larger than those of Figs. 7 and 8, and in a section more vacuole-like cavities may be seen, the number frequently being twelve or more.

At this stage of development there are also present in the cytoplasm many rounded bodies that resemble leucoplasts, together with very small and densely staining granules mentioned in a foregoing paragraph (Figs. 7, 8). These bodies differ in staining capacity from leucoplasts of the same size; they are larger and more numerous than the starch grains revealed by the iodine test made at the same stage of development of the endosperm, and they stain a light brown, or pale yellowish colour, with the technique here employed. Their colour is almost that of the unstained section, probably the colour imparted by the osmic acid used in the killing fluid. They contrast strongly with the black granules in the vacuole-like cavities. It is probable that these bodies are concerned with the synthesis of oil, and we shall return to them in a later paragraph.

In a careful study of the vacuole-like cavities, containing the densely staining granules, in endosperm cells representing different stages of maturity, the behaviour of these bodies can be readily followed. In Figs. 9 *a*, 9 *b*, are shown two such cavities of different sizes, but containing many granules that are small and uniform in their dimensions. In Fig. 9 *c* some of the granules seem to be united into a large globular mass. This phenomenon is perhaps less frequent than that of Figs. 9 *d*, 9 *e*. Here the granules are much larger, especially in Fig. 9 *d*. In Fig. 9 *e* it is clear that these granules are uniting into a larger mass. This explanation is strengthened by what is found in a more mature cell. In an older seed (Fig. 10) the cytoplasm is more uniformly granular, there being fewer leucoplast-like bodies present that stand out clearly and definitely. Instead of vacuole-like cavities, with numerous densely staining granules,

we have generally one large rounded and densely staining body to the vacuole. It frequently happens (Fig. 10) that these large bodies do not lie in a vacuole, while in the same cell the vacuole is conspicuous and much larger than the contained protein mass. In the intercellular spaces, and in the space left in the corners of cells in which some shrinkage has taken place, a homogeneous substance, somewhat brownish in colour, is seen, which in all probability consists of the remains of the oil that was not removed by the alcohol and other reagents used in the processes of embedding and staining (Fig. 10, *x*). Sometimes a portion of this substance finds its way into the cavity surrounding the protein mass. In the more mature cells of the seed the large protein mass (Fig. 9 *f*) gives evidence of becoming angular, and a rounded body is near by, which is doubtless the globoid. Fig. 9 *f* and Fig. 10 were taken from the same section. Angular bodies were not found in the younger stages of development. In endosperm cells of the mature and dry seeds, prepared by fixing and staining, little remains save the angular crystalloids and fragments of the membrane of the aleurone grain. In preparing the endosperm of the dry seed for sectioning and staining, it was necessary to cut it up into small pieces, and, as castor oil is miscible in absolute alcohol, it is not to be expected that any oil would remain in the cells.

The facts brought out in this study seem to point to the conclusion that the aleurone grains in *Ricinus* have their origin in small granular primordia existing in the cytoplasm. Such primordia increase in size and collect in large numbers in vacuole-like cavities. Later they or their generated products fuse to form one or more large masses which, during the final steps in the maturing of the seed, take the form of the well-known crystalloid near which is often found the globoid. The amorphous protein which surrounds the crystalloid of the mature seed, and which is brought into evidence by treating sections of mature endosperm with absolute alcohol followed by iodine, is not to be demonstrated, either in any stage of development as here observed, or in the mature endosperm. There is a possibility that the amorphous protein so called is not real protein, or it is of such a nature as not to be rendered visible by the fixing and staining employed.

There is nothing in the process of development observed here that lends support to the view expressed by Pfeffer that a globoid is the starting-point about which an aleurone granule is formed. Wakker calls attention to small bodies in the centre of the vacuole which shows molecular movements, and which represent the beginning of the future crystalloids. He leaves the impression that each body in the vacuole becomes a crystalloid. He does not mention any fusion of the bodies, and his figures do not help to make the text clearer on this point. As a matter of fact the bodies shown in Figs. 7-9, are so numerous that the conclusion that they unite to form larger masses is imperative.

In a foregoing paragraph attention was called to the many leucoplast-like bodies in the cytoplasm at certain stages of development (Figs. 7, 8), and the suggestion was made that they might be oil plastids or the remains of oil plastids. Inasmuch as castor oil is miscible in alcohol it is not possible to preserve the oil in the preparations. These bodies are more numerous than the transitory starch found in young endosperm cells, and as they do not react towards the stains quite like real starch inclusions, but remain unstained, or take on a pale brownish colour, it seems not impossible that they are concerned in the synthesis of castor oil.

CONOPHOLIS.

For the reason that many bodies are present in the tissues of stem and scale leaves of the phanerogamic parasite *Conopholis americana*, as shown by the ordinary microchemical tests, it was decided to look into the origin of such protein bodies. The meristematic cells of the young organs show the same structure as observed in plants in general, namely, numerous small and densely staining granules or short rods. As the cells become older and pass over into the mature condition, many of these bodies enlarge and stain densely with iron-haematoxylin, as in the case of protein granules in other plants (Figs. 12-14). In a young epidermal cell from the wall of the ovary the primordia of the plastids are numerous and of uniform size (Fig. 12). In cells beneath the epidermis in the same region larger globular bodies may appear sooner than in the epidermis (Fig. 13), but in somewhat older parts—for example, those of the floral envelope—the large globular bodies of Figs. 14 and 15 are as numerous as in cells lying within. From Figs. 14 and 15 it seems clear that the large globular bodies have arisen from the small primordia, as all gradations in size are present. It is not difficult to imagine how conspicuous sections of tissues appear when observed with either low or high magnification if composed of cells like Figs. 14-16. As the cells increase in size and age many of the globular bodies increase in size and stain so densely as to appear a deep black (Fig. 16). These cells taken from the cortex of the flower pedicel reveal the fact that there are also leucoplasts, though in smaller numbers. An iodine test of fresh and alcoholic stems of *Conopholis* demonstrates the presence of starch here and there in the older, as well as in younger, tissues of the cortex especially. In the cells represented in Fig. 16 the starch grains, both simple and compound, are aggregated sometimes about the nucleus and sometimes in the corners or other parts of the cell. They are small and relatively few in number as compared with the protein granules. The latter vary greatly not only in size but in staining capacity. Some are uniformly black in appearance, whilst others, especially among the smaller, have a dense centre and a paler periphery. Certain of these are almost colourless, with a sharp contour and a dense spot in the centre.

In older cells the protein granules become very large, and the impression is that these result from the fusion of smaller ones (Figs. 17 *a* and 17 *b*). In old cells, as in parts of the floral envelope and scale leaves, these larger bodies seem to be hollow, possessing a dense dark periphery and a colourless centre (Fig. 18). They are as a rule not globular, but elongated and irregular in outline. These bodies give a protein reaction with the usual microchemical tests for this substance in plant cells.

Conopholis is a complete parasite, utterly devoid of chlorophyll, living upon the roots of forest trees in this locality, and the writer was much surprised, when studying the preparation from which Fig. 16 was taken, to find starch grains. At first he was in doubt as to the accuracy of the observation, but an iodine test of freehand sections of both fresh and alcoholic stems left no ground to question the presence of starch. The leucoplasts in this parasite are able, therefore, to synthesize starch from elements obtained from its host.

DISCUSSION.

In an earlier publication (Mottier, 1918) the writer traced the origin of leucoplasts and chloroplasts from small granular and rod-shaped primordia, which had been generally referred to as mitochondria, chondriocents, or chondriosomes. He maintained furthermore that these primordia were permanent organs of the cell, and emphasized the view, probably expressed first by A. F. W. Schimper (1880), that leucoplasts and chloroplasts were morphologically alike.

It was further pointed out that, in the same cells along with the primordia of leucoplasts and chloroplasts, other bodies were present, similar in form, size, and appearance to these primordia, which did not develop into leucoplasts and chloroplasts. For convenience and clearness the term chondriosome was applied to these rods and granules that did not develop farther. The same term was applied to similar rods and granules found in cells of certain Liverworts, such as *Anthoceros* and others. The writer did not attribute to these bodies any specific function. In the Summary (l. c., p. 112) he suggested that the bodies which were designated as chondriosomes, for convenience, might be the primordia of plastids.

Guilliermond (1919) seems to take exception to the above use of the term chondriosome, claiming that the case of the Liverwort cited was a special case. He emphasized the view that granular and filamentous mitochondria and chondriocents are different forms of the same structures, and that they are of equal significance (l. c., pp. 242-3). As a matter of fact my use of the term chondriosome in the Liverwort applied to both granular and slender rod-shaped bodies that did not develop into chloroplasts, just as in the case of other plants. In later publications (1920) Guilliermond seems to have changed his view somewhat, as he admits that

in the pollen mother-cells of *Lilium candidum*, for example, there are mitochondria which do not become leucoplasts. He seems to prefer that all small granules and rods in the cytoplasm be called mitochondria and chondriocots, whether they do or do not develop into known plastids.

Emberger (1920) recognizes in the cells of the developing sporangium of the fern plastids and mitochondria, stating that, in the cells of the maturing sporangium, chloroplasts revert to mitochondria.

In the description of the origin of the plastids here under consideration in this paper, I have not used the term mitochondria or chondriosome to designate any body in the cell. In meristematic cells of most plants, certainly in all examined by the writer, including fungi, many very small and densely staining granules and rods are to be readily observed. This statement seems to apply equally well to cells of certain animals. It is to be regretted that too many observers have been content to demonstrate in cells of both plants and animals the mere presence of these small bodies, referring to them merely as mitochondria or chondrosomes, without any effort to determine what such bodies might develop into. In the present state of our knowledge, there is certainly nothing to be gained by the mere demonstration of small objects under the names mitochondria, chondriocots, &c. Inasmuch as these terms have been applied to different objects in both animals and plants, it is questionable whether they should continue in use.

In addition to leucoplasts and chloroplasts having their origin in definite primordia, which are permanent organs of the cell, the writer feels justified in adding protein plastids and perhaps also oil plastids.

In the endosperm of *Zea Mays* there seems to be no doubt that the protein granules, known as aleurone grains in the outer layer, are derived from definite and pre-existing primordia that first appear as small granules or short rods. As these bodies elaborate the protein they increase in size, many assuming large proportions. They stain definitely and densely with protoplasmic stains. In *Zea* the writer is not prepared to state whether two or more of these protein granules fuse into larger masses as the seed matures.

In *Ricinus* the protein granules take their origin likewise in definite primordia. These generally aggregate in vacuole-like cavities, and, as the cells mature, the product of their elaboration unites into larger masses, known as the aleurone grains. Within these large masses a part of the protein takes on the form of a crystal or crystals near which appears the body known as the globoid. The amorphous protein surrounding the crystalloid does not take the stain in the preparations. This aggregation of the protein plastids in cavities may be correlated with the presence of oil which is elaborated simultaneously in the same cells.

The writer has in a foregoing paragraph called attention to leucoplast-like bodies, which, however, did not react to the stains like normal

leucoplasts. It is suggested here that these objects represent oil plastids or what remains of them. The oil, being miscible in absolute alcohol, has, of course, been removed in large part from the cells. In *Ricinus* there are no elaioplasts present such as has been described by Beer (1909) and earlier investigators. These bodies seem to be more numerous than the transitory starch grains appearing earlier. It may be asked within reason whether leucoplasts are able under certain conditions to synthesize oil as well as starch.

In the parasite *Conopholis*, the large rounded bodies giving a protein reaction can be traced with precision to pre-existing primordia, distributed in the cytoplasm as very small granules or rods.

In view of the question raised in the paragraph preceding the last it may not be out of place to call attention to a marked similarity between leucoplasts and chloroplasts. Schimper (1880) was perhaps among the first to point out concisely this similarity. The following is a translation of his summary on the subject: 'The results of this brief study show that the deep chasm hitherto supposed to exist between the starch-formers in assimilating (chlorophyll-bearing) and in non-assimilating cells does not, in fact, exist. In cells free from chlorophyll there are definite organs which generate starch, and these organs are none other than undeveloped chloroplasts (*Chlorophyllkörner*), which under the influence of light are able to develop into the latter. On the other hand, chlorophyll grains are not always organs of assimilation merely, but they may, in conducting tissues and in cells which contain reserve material, function as starch-formers in the non-assimilating cells; they produce starch from assimilated materials supplied by other parts of the plant.'

As applied to certain parts of many plants Schimper's conclusion is correct, at least as far as known. In this connexion it is well to note certain differences between leucoplasts and chloroplasts, bearing in mind the fact that these generalizations may not apply with equal precision to the chloroplasts in all plants. Leucoplasts and chloroplasts originate in definite primordia which cannot be distinguished one from the other. As Schimper pointed out, under certain circumstances one seems to be able to change into the other. Both make starch, but chloroplasts can elaborate this substance out of carbon dioxide and water, while leucoplasts make starch from the products of photosynthesis. Mature chloroplasts possess the power of growth and division in adult cells. They manufacture and give up their starch successively and repeatedly. In so far as the writer is aware leucoplasts cannot divide after reaching maturity. When they have elaborated their starch they pass over in that form into the permanent vegetative cells. Having reached the adult stage they cannot be rejuvenated.

While these statements seem to be true as applied to many plants,

yet the possibility is not excluded that leucoplasts may elaborate bodies other than starch. All materials elaborated by plastids are derived primarily from the mineral salts taken from the soil and from the products of photosynthesis. Even in parasites devoid of chlorophyll, as *Conopholis*, where leucoplasts containing starch inclusions are present, the materials used by the leucoplasts came originally from the photosynthetic products of the host.

In a voluminous publication, profusely illustrated, Guillermond (1919) endeavours to show that, in the cells of the flower of *Iris germanica*, *Tulipa*, and in flowers of some other plants, the pigments xanthophyll and carotin arise from plastids whose primordia are granular or rod-shaped bodies which can be recognized in the living cells with precision.

Guilliermond (1920, 2) concludes furthermore that, in the epidermis of the young leaves of *Iris germanica*, vacuoles are formed by the hydration of a pre-existing substance in the cells in the form of mitochondria. This explanation as to the origin of vacuoles suggest the tonoplast theory.

The earlier botanists, as Pfeffer, de Vries, and others, were inclined to look upon the cytoplasm, and the cell-sap especially, as a sort of mother fluid from which, with the exception of starch, reserve products, together with anthocyanin and the products of chromatophores, were separated as a physio-chemical precipitation. The trend of research at present is towards the view that these substances in the cell are the synthetic products of permanent organs or plastids. These syntheses are, of course, supposed to be built up in accord with physical and chemical laws. A vital force is not assumed. But it is not admitted that these physio-chemical laws have been definitely formulated, in many instances at least. No one is prepared to tell us what chemical process is involved in the division of a chromosome or of a chloroplast. It has been seen that the function of a leucoplast may, under circumstances, be very similar to that of a chloroplast, and from the standpoint of function a leucoplast need not be greatly different from an oil plastid. The synthesis of starch and castor oil are processes that do not demand a widely different chemical activity, and it may not be bad chemistry to imagine that a leucoplast may, under certain circumstances, be able to synthesize castor oil as well as starch.

SUMMARY.

In addition to leucoplasts and chloroplasts, protein and probably oil bodies owe their origin to plastids whose primordia are permanent organs of the cell, existing in the cytoplasm as densely staining rods or granules.

In *Zea Mays* the primordia of the protein plastids are chiefly small, rounded bodies distributed in the cytoplasm. These increase in size, becoming relatively large and very numerous, especially in the outermost layers of endosperm, the well-known aleurone layer. Small protein

granules arising in the same manner occur also in cells beneath the aleurone layer. These seem to be more numerous in sweet corn.

The protein granules in *Ricinus* arise also from pre-existing granular or rod-shaped primordia. They aggregate in large numbers in vacuole-like cavities, and their combined products unite in these cavities to form the large aleurone grains of the mature seed. As the seed matures a part of the protein, at least, forms the crystalloid or crystalloids near which the globoid arises. There was nothing in this study to indicate that a globoid was a centre for the formation of the aleurone granule as claimed by Pfeffer.

The protein bodies in the parasite *Conopholis* arise from primordia in the same manner as in the endosperm of *Zea*.

It is possible that the oil in the seeds of *Ricinus* owes its origin to oil plastids. The author is not able at the present writing to speak with full assurance on this point. He is of the opinion that drops of oil and fat in the cytoplasm are the synthetic products of oil plastids.

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EXPLANATION OF PLATE XV.

Illustrating Professor Mottier's paper on Certain Plastids, with Special Reference to the Protein Bodies of *Zea*, *Ricinus*, and *Conopholis*.

All figures were drawn from permanent preparations, with the aid of the camera lucida.

Figs. 1-5. *Zea Mays*.

Fig. 1. Starchy corn. A cell from the outermost layer of the young endosperm. The granules of varying sizes in the cytoplasm represent the primordia of plastids. $\times 1,200$.

Fig. 2. Starchy corn. Two cells of the aleurone layer from an older grain of corn with the next three cells beneath in a radial direction. The young protein granules, aleurone, are now very evident. Among them in the cytoplasm are numerous densely staining granules. In the first two cells beneath the aleurone layer at this stage few protein granules are present. $\times 1,200$.

Fig. 3. *Golden Bantam*. A cell of the aleurone layer, from a much older grain of corn. The cell-wall is very thick. Among the very large aleurone granules are many smaller, representing a complete gradation to the primordia. $\times 1,200$.

Fig. 4. Three cells lying in a radial direction beneath Fig. 3. In all three are numerous protein granules of a more nearly uniform size. In the first cell no starch grains are present, a few appearing in the second; in the third and larger cell a number of compound starch grains are present among the numerous protein granules. $\times 1,200$.

Fig. 5. Starchy corn. A cell lying deeper in the endosperm, of the same section from which Fig. 2 was drawn. The numerous simple starch grains are aggregated about the nucleus. In the groundwork of the cytoplasm numerous small and densely staining rods and granules are also present. $\times 1,200$.

Figs. 6-11. *Ricinus*.

Fig. 6. A cell from the periphery of the endosperm with several vacuoles containing only cell-sap. In the cytoplasm are larger rounded bodies and smaller rods and granules, the primordia of plastids. $\times 1,300$.

Figs. 7 and 8. Cells lying deeper in the endosperm. In the centre of each cell lies the nucleus. About midway between nucleus and cell-wall are arranged with considerable regularity the vacuole-like cavities, in each of which are contained numerous rounded and very densely staining bodies, the primordia of the future protein masses or aleurone grains. In the cytoplasm are also rounded bodies resembling leucoplasts, and staining lightly. They may be oil plastids. Among them are very small densely staining rods and granules. In Fig. 8 the bodies in the vacuole-like cavities are larger. Figs. 7 A, 7 C, two cavities of different size, and containing respectively granules of different size. $\times 1,300$.

Figs. 9 a-o. Successive steps in the formation of a more mature aleurone mass. f was taken from the same endosperm as Fig. 10. $\times 1,300$.

Fig. 10. The smaller bodies have united to form the more mature protein masses. Each usually lies in a definite cavity, but all do not. The leucoplast-like bodies are only faintly visible. At *x* is an almost homogeneous substance which has accumulated in the space left by a slight shrinkage of the cytoplasm. A similar substance sometimes accumulates in the cavity about the protein mass. This homogeneous substance represents probably the remaining oil. $\times 1,300$.

Fig. 11. Starch grains from the periphery of the endosperm. $\times 1,300$.

Figs. 12-18. *Conopholis americana*.

Fig. 12. Epidermal cells from meristematic region. The small bodies in the cytoplasm are the primordia of plastids. $\times 1,300$.

Fig. 13. Meristematic cell deeper in the tissue. A number of globular protein granules are present. $\times 1,300$.

Figs. 14 and 15. Older cells in which the protein granules are much larger. $\times 1,300$.

Fig. 16. Portions of three older cells from the cortex of the stem. Starch grains are aggregated about the nucleus or elsewhere in the cells. The very numerous protein granules of different sizes stain a dense black with the iron-haematoxylin. $\times 875$.

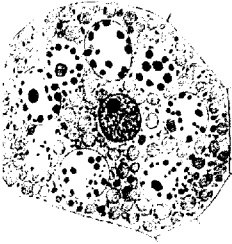
Figs. 17 *a*, 17 *b*. Two large protein masses from an older scale leaf.

Fig. 18. A section of a large protein mass in an adult cell. This body* seems to be hollow. $\times 875$.



Mottier-plastids.

MOTTIER-PLASTIDS.



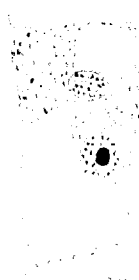
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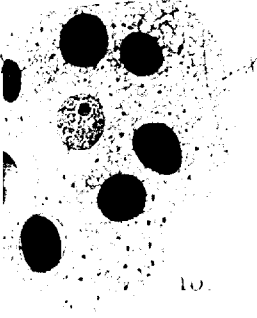
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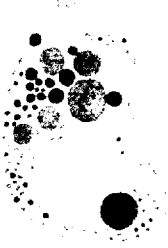
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A Cytological Study of Pollen Development in *Lactuca*.

BY

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With Plates XVI-XIX.

INTRODUCTION.

IN the following account we propose to describe the cytological phenomena of meiosis in the pollen development of cultivated lettuce, *Lactuca sativa*, also the history of the tapetal cells and the later development of the pollen grains. Cultivated lettuce is generally supposed to have been derived from *L. Scariola*, L., and has been in cultivation for over two thousand years. But as *L. Scariola* occupies the whole of the Mediterranean region and Eastern Asia, it probably contains a number of micro-species, from one or more of which the cultivated varieties were originally derived. Some of the peculiarities of pollen development suggested a possible effect of this long period of cultivation. We have, therefore, compared the cultivated lettuce with the two wild species *L. Scariola*, L., and *L. muralis*, Fres., in all points in which an effect of cultivation on *L. sativa* seemed possible.

The earlier stages of the work were completed, and a preliminary account of the results published by the senior author (Gates, 1920). All the later preparations and the whole of the drawings have been made by the junior author, and all stages of the development have been critically studied by us both. All of the cytological material was obtained from the John Innes Horticultural Institution, Merton, through the kindness of the Director, Dr. W. Bateson, F.R.S., who is making a genetic study of certain cultivated lettuces and wild species of *Lactuca*.

(Annals of Botany, Vol. XXXV. No. CXXXIX. July, 1921.)

B b

The material originally collected in 1919 came from a sowing of commercial seed of Sutton's 'Dwarf Perfection'. This is essentially a Cos lettuce and habitually throws 1 to 2 per cent. of 'rogues', the rogue resembling a Cabbage lettuce. The type, however, when carefully selfed, we are informed by Dr. Bateson, breeds true as far as present experience goes, some five hundred offspring all remaining true to type. But the rogues 'throw a profusion of forms, ranging from a small percentage of good Cabbage lettuces, through miscellaneous mongrels, to an occasional plant approaching Cos, but I doubt if a true Cos, or even a true Dwarf Perfection, came in some hundreds of them' (Dr. Bateson in letter). There are difficulties with the view that the rogues are cross-bred, and these preliminary results of Bateson are referred to here as indicating all that is at present known concerning the relationship of the type and the rogue. Cytologically, the type and the rogue are very similar, and no certain differences have been established between them.

The most satisfactory fixing fluid was found to be 1 per cent. chromo-acetic acid solution, and Heidenhain's iron-alum-haematoxylin gave the most sharply defined and satisfactory staining results for most purposes. The sections were cut chiefly 10μ in thickness. The figures are all of cultivated lettuce, and no distinction is made between the type and the rogue except in the explanation of plates at the end of the paper.

EARLY HISTORY OF POLLEN MOTHER-CELLS AND TAPETUM.

In a longitudinal section of the buds of lettuce, a very small number of pollen mother-cells appears in each loculus. Numerous counts in all the species indicate the presence of from fifteen to twenty mother-cells in each loculus, from which usually about sixty pollen grains develop. Moreover, a striking feature of all three species is the loose arrangement of the cells in even the earliest pollen mother-cell stage. The separation of the cells begins in the archesporium. As shown in Figs. 1-4, not only are the pollen mother-cells frequently separated from each other even before synapsis, but they usually lie free from the tapetal cells, and the latter are for the most part more or less completely separated from each other. This last condition is so different from the usual condition in flowering plants in which the tapetum forms a compact and continuous layer of cells, that it was at first thought to be possibly a result of long cultivation. But comparison with the two wild species showed this condition to be equally characteristic of them, and so probably of the genus as a whole.

Another peculiar feature which is associated with the loose arrangement of the tapetal cells is the fact that a variety of transitional stages occur between tapetal cells and pollen mother-cells. During the period when the mother-cells are in synapsis and the postsynaptic spireme stages, the tapetal

cells vary greatly in appearance, as shown in Figs. 2-4. Individual tapetal cells frequently break down even during presynaptic stages (see Fig. 1). They are at this time uninucleate. The tapetal cells become binucleate by a mitotic division at about the time of the onset of synizesis in the pollen mother-cells, or even a little earlier. This division may be somewhat delayed (Fig. 2), but the cells are usually all binucleate by the time synizesis is complete. The second mitotic nuclear division, which makes the tapetal cells quadrinucleate, occurs at the end of the period of synizesis or when the pollen mother-cell nuclei are just coming out of synizesis and forming a loose spireme. These nuclear divisions are usually simultaneous throughout a loculus.

Among the numerous types of tapetal cells, many of them remain, at least for some time, in the binucleate condition. Occasionally, crowding of the spindles or failure of one nucleus to divide will result in a trinucleate cell. The variations and later history of these peculiar tapetal cells will be considered below.

SYNIZESIS AND SYNAPSIS.

In this paper the term synizesis is adopted for the tightly contracted phase of the nucleus, following the usage which has become customary in the literature of animal cytology, while the term synapsis is applied to the whole period from the beginning of contraction in the nucleus, through the various stages of spireme formation until the spireme segments into chromosomes.

Synizesis thus represents the height of the synaptic contraction, which takes place in the earlier stages of synapsis. The essential feature of synapsis has always been considered to be the pairing of elements of maternal and paternal origin. The more recent discovery that the maternal and paternal chromosomes frequently show a tendency to be paired in the somatic nuclei of many plants and animals has rendered it very doubtful whether synapsis is ever concerned at all, in organisms in which telosynaptic reduction takes place, in actually bringing about the paired association. This phase of the subject will be considered later.

Fig. 5 represents a presynaptic pollen mother-cell in which the nucleus is occupied by a uniformly distributed reticulum of fine, anastomosing threads.* This threadwork is only slightly thickened at the nodes, and there is no trace of prochromosomes in the resting stage. Almost invariably a single large nucleolus is present, but very occasionally two have been observed. The first indication of the onset of synizesis is the appearance of a clear space at one side of the nucleus, accompanied by a thickening of the reticulum threads (Fig. 6). Many mother-cell nuclei have been seen in this condition where other nuclei in the same section show later stages of synizesis, as represented in Figs. 7-11. The cytoplasm of such cells is properly fixed and not unduly contracted.

It therefore seems clear that the appearance of this hyaline area, which is always at one side of the nucleus and in contact with the nuclear membrane, is the very beginning of synizesis. Sometimes at this stage the nuclear membrane appears to be ruptured as though from internal pressure, leaving a small clear area at the point of rupture extending into the cytoplasm. The cause of this condition is obscure. In Fig. 6 the reticulum appears to have been drawn away from the nuclear membrane. Figs. 7-10 illustrate further advance of this contraction. The reticulum is gradually withdrawn from the nuclear membrane, sometimes uniformly at all points so that the contracted mass comes to lie in the centre of the nucleus, but more usually to one side, where it still remains in contact with the nuclear membrane.

In the early stages leading to synizesis, represented by Figs. 7-10, while the nuclear reticulum is undergoing varying degrees of contraction, it is at first usually surrounded by an extremely delicate membrane, probably osmotic in character and much thinner than the nuclear membrane, which enables the contracting threadwork to preserve a more or less perfectly spherical form. Delicate threads may be present, connecting the reticulum with the nuclear membrane (Fig. 9), or no such threads may be visible (Fig. 8). These threads may be secondary in origin. When present, they apparently tend, by their attachment to the nuclear membrane, to give the contracting reticular membrane a wavy outline. Fig. 10 represents a slightly different condition which is of more frequent occurrence. Here the delicate membrane surrounding the reticulum has apparently never been complete in itself, being completed by a portion of the nuclear membrane to which the threads of the reticulum remain attached, though obviously drawn out from their original position.

In both these cases, whether the reticulum becomes completely free from the nuclear membrane or remains attached by one portion, the necessary conditions are present for the functioning of an osmotic membrane enclosing the reticulum. Exactly how this membrane arises we do not know. It appears to be a precipitation membrane which is laid down after the contraction of the nuclear reticulum has begun to withdraw the latter from contact with the nuclear membrane, and hence appears where the reticulum comes in contact with the karyolymph. It is possibly first formed in contact with the nuclear membrane, and then gradually withdrawn from that membrane. In *L. Scariola* it is usually more conspicuous than in lettuce. It is owing to this membrane that the reticulum still retains in most cases its spherical outline, the evenness of which is an indication of the presence of a limiting membrane. This membrane has in fact been clearly observed in a large number of nuclei at this stage and can be readily made out from Figs. 8 and 10.

The possible manner of functioning of such an osmotic membrane

is clear. If it is semipermeable, and the clear area of karyolymph surrounding the reticulum contains more strongly osmotic substances, then progressive contraction of the network would take place as water is withdrawn into the clear area between this membrane and the nuclear membrane. The excellent fixation of the cytoplasm in these cells makes it very difficult to suppose that these phenomena result from the treatment.

But it is evident that the presence of an osmotic membrane surrounding the reticulum will not entirely account for the contraction, because numerous stages of contraction have been seen where no such membrane is present. In view of the statement made by Lawson (1911), that the apparent contraction is really due to the sudden growth of the nucleus and is not a true contraction, a series of measurements were made beginning with the resting stage and ending with the postsynaptic spireme. These measurements showed conclusively that, in *Lactuca* at least, there is a real contraction of the nuclear content. There is at the same time a steady increase in the nuclear volume, and the two phenomena appear to be more or less simultaneous, though the contraction may begin first, and the nuclear expansion usually continues after the reticular contraction is completed. The suggestion of Davis (1910), that contraction is due to shortening and thickening of the threads of the network previous to their transformation into a spireme, also deserves consideration. Comparison of the reticula in Figs. 8-10 with the resting nucleus shows clearly that the threads in the former take up considerably less space, but it has been impossible to find clear evidence that the threads are individually noticeably thicker. A membrane surrounding the contracting reticulum might then function as an osmotic aid to contraction, but that contraction can take place in its absence is shown by such figures as 7. At times, as in Fig. 7, the transformation of the thread into a spireme occurs prior to the formation of the true synaptic knot.

As the contraction proceeds it is often accompanied in its later stages by a rearrangement of the threads of the reticulum to form a more or less continuous spireme. It is impossible to determine the exact nature of this rearrangement of threads. It appears to be a process quite distinct from the contraction, and in many cases (Fig. 7) the rearrangement is already taking place before the contraction has proceeded very far. In such cases the nuclear membrane is frequently not intact, and there is no evidence of a precipitation membrane. Nuclei in this condition at first suggest artifact, but the cytoplasm is well fixed, and as contraction of the nuclear content takes place in different directions in adjacent cells there is no reason for attributing it to the fixing fluid. The stages represented by Figs. 8-10, compared with Fig. 7, indicate that the amount of contraction of the reticulum may perhaps be conditioned to some extent by the presence of a precipitation membrane.

Even though possibly a certain amount of derangement of the nuclear content takes place during fixation, yet it is clear that these nuclei represent a peculiar physiological condition of the nucleus at the onset of synizesis. This is indicated by the manner of their occurrence, for usually the cells of a locus will all show approximately the same condition, while adjacent loculi in the same section will be in other stages of development. Thus Figs. 8 and 9 are from one locus, and Figs. 7 and 10 from an adjacent one. Typical synizesis, and later postsynaptic spireme stages (Figs. 11-12), may also occur in the same slide or in different flowers of the same section. Since synizesis itself and the later spireme stages are universally recognized as normal developmental stages, the same status must be accorded to the transition stages represented by Figs. 7-10.

All the above stages are exact duplicates of conditions described by one of us in *Oenothera rubrinervis* (Gates, 1908, see Pl. I, Figs. 12-15), and in *Oc. gigas* (Gates, 1911, see Pl. LXVII, Figs. 2-6). Fraser (1914) has also figured in *Vicia Faba* (Figs. 2, 3) conditions very similar to our Figs. 6-10. In *Oenothera* there was evidence of expansion or growth of the nuclear membrane at this time, as well as contraction of the nuclear contents.

Lawson's (1911) view that in synizesis there is 'a great accumulation of sap within the nuclear cavity' which 'causes great osmotic pressure', producing a distension of the nuclear membrane, is quite inadequate to explain the phenomena observed. His results with *Smilacina* as to the absence of any contraction of the nuclear content at this period have not been confirmed in any other plant, and McAllister (1913) has since figured typical synizesis in this genus. Consideration of all the results show that while there is a certain amount of expansion or growth of the nuclear membrane both in *Oenothera*, *Smilacina*, and *Lactuca*, there is also a notable contraction of the nuclear reticulum at the same time. In *Lactuca* and also in *Oenothera* there is clear evidence of a delicate membrane surrounding the reticulum in the early stages of its contraction.

In *Lactuca* the evidence for contraction of the nuclear reticulum is conclusive, but there is also clear evidence of growth of the nucleus during the later stages of synizesis (Fig. 4). This growth and a number of other size relationships are shown in the table of comparative measurements given below.

Each figure given is the average of twenty-five measurements, or in a few cases ten measurements. The range of variation was small except in the synizesis stage of the cells in *L. Scariola* marked (*). The number of measurements, while not large, was found to be sufficient for the present purpose. Reading down the vertical columns, there is seen to be a steady growth in the size of the mother-cell from the presynaptic to the postsynizesis stage. The same is true of the nucleus during this period. The

difference between the synizesis and postsynizesis measurements in *L. Scariola* as regards size of both cells and nuclei is probably not significant, and this would appear to indicate that cell growth ceases earlier in this species than in the lettuce.

TABLE I. *Dimensions of Cells and Nuclei in Lactuca.*

	<i>Lettuce—Rogue.</i> Average Diameter.	<i>L. Scariola.</i> Average Diameter.
	mm.	mm.
<i>Cell.</i>		
Resting nucleus	19.7	17.7
Beginning of synizesis	20.3	17.8
Synizesis	21.8	23.4*
Postsynizesis	26	22.5
<i>Nucleus.</i>		
Resting stage	11.4	10.1
Beginning of synizesis	12.8	13.4
Synizesis	14.2	14.9
Postsynizesis	15.2	14.6
<i>Reticulum.</i>		
Early in synizesis	7.7	7.4
Synizesis	6.5	7.4

That in synizesis the reticulum undergoes contraction to approximately half the diameter of the nucleus is shown by comparison of the last two lines of the table with the preceding lines. Needless to say, all the nuclear measurements were taken from nuclei which had not been cut, so that the whole of the contracting reticulum or synaptic knot was present. With nuclei of such small size this is easily done when the sections are 10 μ thick.

Hitherto, synizesis has only been reported from spore mother-cells in plants and in the primary spermatocytes and oocytes of animals. This has emphasized the purely morphological or developmental aspects of the process. It evidently represents a unique and critical stage in the life cycle of any sexually reproduced organism, having been observed even in such low organisms as the Myxomycetes (Olive, 1907). But like every other developmental stage, it must be produced by antecedent physiological conditions which bring it about. These conditions usually arise only in spore mother-cells. But a peculiarity of *Lactuca*, as already pointed out, is the presence of a variety of intergrades between pollen mother-cells and tapetal cells. The presence of such intergrades is believed to account for the occurrence of synaptic phenomena in cells which are essentially tapetal cells.

Fig. 74 shows a binucleate tapetal cell with its nuclei in synizesis. That it is a tapetal cell and not a binucleate microspore mother-cell, such as Fig. 15, is shown by its position. It forms a part of the tapetal lining of the loculus, while the pollen mother-cells are in the postsynaptic spore stage. The adjacent tapetal cells do not show this condition, except one trinucleate cell (Fig. 82) on the opposite side of the loculus. The central

nucleus of this cell has probably been formed by the close approach to each other of the inner ends of the two spindles present during the second mitosis, leading to the coalescence of the two inner daughter nuclei into one at the time of their formation. This frequently happens in tapetal cells owing to the crowding of the spindles, so that a number of the tapetal cells are trinucleate after the second mitosis. In Fig. 8a this central fusion nucleus is in a somewhat later synaptic condition than the two end nuclei. The fact that synizesis occurs in these cells cannot, we think, be attributed to the fixation, but must rather be an indication of a peculiar physiological condition of these nuclei.

Fig. 11 shows a mother-cell in the typical synizesis stage. Even with the most careful treatment and differentiation, the synaptic knot is invariably so dense that it is impossible to trace the spireme thread for any distance. Soon, however, threads begin to appear at the margin, and the whole mass is seen to be composed of looser and thicker threads, until a stage is reached such as shown in optical section in Fig. 12. The spireme emerges as a thick uniform thread which is apparently more or less continuous throughout its length, though occasionally free ends may be observed. No trace of any parallelism of the threads has been found in these early stages, the thread appearing uniform and unsplit, even when weakly stained. As the spireme spreads out through the nuclear cavity after synizesis, definite loops very soon make their appearance, as seen in Figs. 18-23. At first it is impossible to make an accurate count of these loops. Figs. 18-20 show early stages in the process of looping. The sides of the loops come to be more or less parallel, and it will be seen that the two sides of a loop are also beginning to twist round each other. This latter feature becomes more and more evident in the later stages. As the process of looping continues, it is possible to make a rough count of the number of these loops, and it is found to be approximately nine, i.e. the number corresponding to the gametic number of chromosomes (Figs. 23-29). Finally the loops gradually become detached from each other. There is at this time no definite polarization of the spireme loops. They do not radiate regularly from the centre of the nucleus, nor are they polarized in any other way corresponding to the 'bouquet' stage which occurs in the presence of a centrosome; also no definite second contraction stage has been observed, such as occurs in various other plants. The loops continue to be well distributed through the nucleus.

While the spireme, now a pachynema, is for the most part uniform in appearance during this period, it is by no means universally so. As the figures show, thinner threads are occasionally found connecting thicker portions of uniform thickness. Also some portions may have a beaded appearance owing to the alternation of lighter and darker areas along the threads. But none of these appearances when critically studied permit of

the interpretation that they have anything to do with a possible conjugation of parallel threads. While it is difficult to affirm from direct observation that no such parasynaptic conjugation of parallel threads takes place during synizesis, yet we have found no evidence whatever which would bear such an interpretation, and we believe the nature of the later stages excludes the possibility of its occurrence. The thread might of course become double at this time through a split, as has been described in various forms, but in *Lactuca* we have found no indication of a split during this period. The thread remains persistently single as it becomes progressively shorter and thicker.

The segmentation of the spireme into separate loops is a progressive affair, different loops becoming gradually detached. Figs. 23-25 show stage in which a few loops are free while the others are still connected in continuous spireme. At this time the characteristic twisted appearance of the loops becomes yet more marked. The detached loop in the lower part of Fig. 23 shows this very clearly. That certain chromosome pairs frequently become detached from the spireme and undergo precocious condensation was shown in the case of *Oenothera* (Gates, 1908, Pl. II, Figs. 20-24), but in that genus the chromosomes are so short and thick that there is never any twisting of the members of a pair about each other during the condensation period. Twisting of the chromosomes about each other, with subsequent breaking at the nodes, is therefore apparently excluded. Its absence in *Oenothera* may perhaps be genetically significant, since it is known that in that genus the characters in crosses usually have a strong tendency to remain together in groups instead of showing either free assortment or 'crossing over'.

Fig. 30 represents a later stage in lettuce, where all the loops have become free and the twisting has reached its climax. The loops clearly correspond in number to the x number of chromosomes, and one is consequently led to the conclusion that each loop represents a pair of homologous chromosomes. The later fate of the loops, which will be described below, completely confirms this view. The only satisfactory interpretation of such an arrangement would appear to be that the spireme is made up of the full somatic number of chromosomes, which are arranged end to end in pairs, the members of which are alternately of maternal and paternal origin. The two members of each pair then bend round and form a loop, so that the parental chromosomes of each pair come eventually to lie side by side but still connected at one end. This is very similar to the course of events in *Oenothera* (Gates, 1908, Pl. II, Figs. 20-26).

The sides of the loops in lettuce then twist around each other. Usually this results in two or three or more turns (Fig. 30), but sometimes the overlapping is only at one point giving a figure 8 (Fig. 31). Such figures of course are familiar enough in the cytological literature, having been

observed in various plants and animals for many years. Their significance as a possible basis for genetic crossing over will be discussed later, and important differences from the chiasmata described by Janssens (1909) will be pointed out. Actual proof that this twisting of the homologous chromosomes about each other is followed in any case by an exchange of segments is extremely difficult to get. Inspection of the figures shows that the wrapping of the chromosomes about each other is much more intimate in some cases than others. Quite probably in many cases no exchange of segments follows, but such cases as Figs. 33 and 49 appear to furnish definite evidence in favour of the occurrence of an interchange of loops.

During this period the nuclear membrane is often extremely thin or quite invisible (Figs. 18-23). That the chromosome pairs differ constantly in length in these early stages of their formation, as in the later stages, is clearly shown by such figures as 23 and 28. This differentiation in length makes it possible to show that the pairing is between homologous chromosomes of similar length, and therefore of maternal and paternal origin respectively.

DIAKINESIS.

The chromosome pairs, lying almost invariably side by side, and usually more or less twisted about each other, undergo progressive condensation to form the definitive chromosomes. During the later stages of this condensation the chromosomes become very compact, and finally (Figs. 37-48) appear as straight or curved rods of different lengths, usually showing no indication of their bivalent nature, except in some cases by a fork at one end. It is possible that the swelling produced by the fixing fluid may obliterate the line of separation between the two halves of a bivalent chromosome more completely than in the living condition, but in any case the relationship between the two halves is exceedingly close at this time. Clear indications of the twisted condition are often observed until the condensation is almost complete (Figs. 33-36). But the only trace of the double nature of the definitive bivalent chromosomes (Figs. 37-48) is a faint longitudinal split or a fork at one end.

The nine bivalent chromosomes form a graded series which can be arranged in a general way in three groups, three of maximum length (four or five times as long as broad), three of intermediate length (about two or three times as long as broad), and three very short and almost cubical.

Frequently one of the first three is longer than the other two, and the remainder form a series which can only roughly be divided into groups. The curved shape of the chromosomes also frequently renders impossible the determination of their exact length. Hence the variations

in relative length from one nucleus to another, as shown by the rows of chromosomes in Figs. 37 *a* to 55 *a*, are probably not so great as they appear.

It is also clear that, as would be expected, it is the longest pairs which invariably show the most marked twisting in the early stages. Examination of the later stages of diakinesis frequently shows the longest or intermediate pairs with a straight longitudinal split. Two possible explanations of this are admissible. Either the loops which were twisted must untwist again, or the pairs must split apart with 'crossing over' of certain segments. While we have no demonstrable evidence that such a rearrangement of segments rather than untwisting occurs, yet figures such as 33, and particularly Figs. 34-36, show that the condition of torsion persists even after the condensation of the chromosomes has progressed far. This gives reason to believe that the subsequent longitudinal split of the bivalent chromosomes will take place across the twists. Moreover, a twist which always untwisted again along the original lines would appear to have no *raison d'être*, whatever are the physical or physiological processes which bring about the torsion.

Occasionally ten chromosomes instead of nine have been counted in diakinesis. This has occurred a number of times in the large number of nuclei at this stage whose chromosomes were counted. The explanation, as indicated by Figs. 37 and 40, is evidently that the two halves of a bivalent chromosome have become separated. In Fig. 40 the two halves of one bivalent are lying parallel and almost separated from each other. The fact that the two bodies marked *a* in Fig. 37 are narrower than the other chromosomes, and are of equal length, makes it clear that they represent the two halves of a bivalent chromosome. Other cases of precocious splitting are indicated in Figs. 35, 39, 42, and 47, in which several of the bivalents are shaped like the V's or X's which are so usual at this stage in many plants. Their relative infrequency here, as well as other facts, indicates that in *Lactuca* the members of the chromosome bivalents are held together exceptionally closely. This is in strong contrast with *Oenothera*, where the attraction which leads to pairing of the chromosomes is exceptionally weak and is a source of irregularities in the distribution of the meiotic chromosomes.

Another irregularity occasionally met with is the presence of only eight pairs (Figs. 46-48). Figs. 45 and 46 indicate how this condition has probably arisen. In Fig. 45 two pairs of bivalents are united by strands of deeply staining material, while Fig. 46 shows seven bivalents of normal length and one extremely long one, formed probably by the union of two long bivalents end to end.

If this structure is composed of two bivalents united end to end it could scarcely have been formed by failure of the spireme to segment

between them, because that would mean that the body is composed of four consecutive segments which remained attached and in which the end segments must have then folded back on the others. This procedure is unlikely, and it seems more probable that the end to end coalescence took place after the chromosomes of two loops had come to lie side by side.

In certain other cases (see Fig. 48) where only eight bivalent chromosomes were present in diakinesis, probably two of the shorter bivalents had fused end to end, but this could not be determined with certainty because two short chromosomes arranged end to end would be indistinguishable from a single bivalent of intermediate length. These fusions will be compared later with others observed on the heterotypic spindle. We have no positive evidence whether the fusions at this stage are temporary or would persist and affect the later chromatin distribution, but probably the latter would be the case.

HETEROTYPIC MITOSIS.

Between the stage of diakinesis and the arrangement of the chromosomes on the heterotypic spindle, the chromosomes become much more condensed. In the heterotypic metaphase they are condensed to such a degree that there remains comparatively little observable difference in length between them, and many of them appear globular or but slightly elongated. The differences in length during diakinesis are not, however, merely a result of different rates of condensation, for the longer chromosomes undoubtedly contain much more chromatin than the shorter. That the differences in length of the chromosomes are constant features is also shown by the somatic mitoses, in which the same graded series of lengths appear (Figs. 63 and 64).

A striking feature of the heterotypic metaphase is the frequent coalescence (more or less complete) of two or four of the chromosome bivalents, so that only eight or seven bodies appear on the heterotypic spindle. Probably over 50 per cent. show fewer than nine bivalents, seven or eight bodies being the most common number, but sometimes (Fig. 57) no more than five bodies are present. This is in cells in which the figure has not been cut, and must be due to a further coalescence of chromosomes. Such cases were the first to be observed, and it was only later that the cause of this apparent diminution in chromosome number was discovered, through the observation of cases in which the nine bivalents were clearly distinct, and also stages in their fusion. Fig. 50 shows an equatorial view with only seven bodies, and Figs. 53 and 54 side views with eight. In Fig. 50 the two bodies (*a*) formed by the end to end fusion of two bivalents can be determined. But in Fig. 54 particularly the coalescence is so complete that there is not the slightest indication as to how it has taken

place. In Fig. 51 is shown a late prophase in which the chromosomes are just being drawn into the equatorial plate. Nine bivalents are present, but two of them are coalesced end to end. There are therefore eight bodies to be seen. Fig. 56 also shows eight, the end to end attachment of two being clear. Cells in the intermediate condition with eight 'chromosomes' appear to be quite as frequent as those with seven.

Bands of darkly staining material frequently occur on the spindle, and are seen connecting some of the chromosomes in Figs. 51 and 54. In Figs. 55 and 56 the whole of the spindle is embedded in a mass of denser and more deeply staining cytoplasm. This feature was found to be typical of the wild *L. muralis*, but these two cells were the only ones found to exhibit the condition in the lettuce rogue and it has not been seen in the type. Fig. 55 represents an equatorial view of the metaphase spindle in which the nine chromosome bivalents are clearly separate.

Figs. 52, 53, 54, 57 show clearly the point of attachment of the spindle fibres, which appears to be terminal in every case. In the last figure the separation of the chromosomes has already begun. All indication of their bivalent nature has at this time completely disappeared. The line of demarcation between the two halves is usually obliterated during diakinesis. This may be partly attributed to the swelling action of the fixing fluid, but even apart from this the two halves of a bivalent must come into very close relationship, especially during the heterotypic metaphase. Again, the coalescence of bivalents at this time cannot be attributed to the fixation, for it occurs in cells where the chromosomes are otherwise loosely grouped. It represents rather a marked tendency to coalescence in which certain of the bivalents appear to be concerned. It is not a mere clumping of the whole chromosome group. The chromosomes are not sufficiently distinguishable at this period to make certain whether two particular pairs of bivalents only are concerned, but the observations lead us to consider that the shorter bivalents are usually concerned, though figures such as 51 and 56 indicate that bivalents of intermediate or maximum length may be involved.

Figures such as 51 and 56 make it certain that the coalescence is an end to end one, at least in the cases which can be clearly determined. We have seen relatively few heterotypic telophases in which the chromosomes could be counted exactly, but these all showed nine chromosomes, indicating that the coalescence is a temporary one on the heterotypic spindle.

These coalescences on the heterotypic spindle are much more numerous than during diakinesis, and frequently two occur in the same cell. From this it follows that while the fusions during diakinesis probably persist into the heterotypic mitosis, fresh coalescences also arise during that division. The possible genetic significance of these phenomena will be considered later.

Fig. 57, which represents an early anaphase, shows the bivalent

chromosomes just beginning to separate. The manner of separation of the halves of a bivalent chromosome is shown exceptionally clearly in Fig. 58. The spindle is uncut and clearly only seven bodies are present—so that this represents a typical case in which two pairs of bivalents have coalesced. Figs. 52 and 54 have already shown that the point of spindle fibre attachment is at one end of the bivalent chromosomes. Obviously, fibres from opposite ends of the spindle must become attached close together to each half of a bivalent, and at the same end of the chromosome. The appearance (see Fig. 57) is as if the contraction of these fibres pulled the two longitudinal halves gradually apart. Two globular pairs of chromosomes are already completely separated, a short and a longer pair still remain attached by a thread at one end, while in the centre, and less clearly at the left side, of the group, two of the longest pairs are being drawn apart. Focusing on the centre pair shows particularly clearly that its central thicker portion consists of the ends (in vertical orientation) which have not yet separated. Thus five of the pairs have already separated, while the other two may each represent two bivalents fused end to end, which would possibly account for their lagging.

Fig. 59 is from a section of a later anaphase in which fifteen or sixteen chromosomes are present. Fig. 60 represents an early telophase in which the full gametic number (9) is seen in each of the daughter nuclei. Several of these chromosomes at least have already undergone the longitudinal split for the homotypic mitosis and appear as X's and V's. The remains of the spindle are still visible, and the nuclear membranes appear incomplete on the side next the spindle. Some of the chromosomes are already beginning to anastomose. In Fig. 61 this process is much further advanced and a cell plate is being laid down, while Fig. 62 represents the interkinesis stages with a loose and heavy threadwork formed in the nuclei. Abnormalities are sometimes found at this time, in which some of the chromosomes pass irregularly towards the poles and frequently give rise to extra nuclei (Fig. 65).

HOMOTYPIC MITOSIS.

We have observed relatively few stages of the homotypic division, which as usual is passed through quickly. In the metaphase, polar view, nine univalent chromosomes were counted in some cases. But one spindle in side view showed only six chromosomes, connected by darkly staining material. It appears that here also coalescence of chromosomes has occurred similar to those of the heterotypic bivalents. But in the absence of more abundant material and with the possibility of the treatment having affected the preparation, we refrain from further interpretation. The stages observed show that in the homotypic mitosis the univalent chromosomes undergo a longitudinal split and separation in the usual way.

FORMATION OF POLLEN TETRADES AND POLLEN GRAINS.

When the reduction divisions are completed, the first evidence of further development in the pollen mother-cell is the beginning of constriction of the cytoplasm at four points placed at equal intervals on its periphery and within the mother-cell wall (Fig. 66). The interval between the cytoplasm and the cell-wall appears to be filled with pale-staining material whose nature has not been investigated. The constrictions of the cytoplasm become progressively deeper until they meet in the centre (Fig. 67) and finally cut up the cytoplasm into four separate masses, the microspores (Fig. 68). As the figures show, this process of constriction of the cytoplasm may take place in the absence of spindle fibres, or it may cut across them before they have disappeared, but in no case were cell plates observed to be laid down on the spindles, the whole process taking place independently of such a structure. Fig. 69 shows a somewhat abnormal tetrad as regards the shape of the mother-cell, and also the way the cytoplasm has been divided up. The mother-cell wall has disappeared and one of the walls has failed to appear, making a central cell with two pollen nuclei. Fusion of these nuclei would give a diploid pollen cell.

It has usually been regarded as an important distinction between the cells of higher plants and animals that whereas in the latter the cytoplasm is segmented after nuclear division by furrowing or cleavage, in plants the new cell-wall is laid down on the spindle. Since Strasburger described the latter method of cell-wall formation in various plants and different tissues, it has usually been assumed to be universal in higher plants. Like so many other distinctions drawn between plants and animals, this is now found to be a general but by no means universal difference. Farr (1916), who refers to the early literature, has described in detail the division of pollen mother-cells in *Nicotiana* by furrowing, exactly as observed in *Lactuca*. He has observed the process both in living and fixed material, and has also described it (1918) in *Magnolia* pollen mother-cells. The same method of furrowing has recently been described by Wanda K. Farr (1920) in *Cobaea scandens*. There is no doubt that it will be found to occur in a large variety of plants, though by no means to the exclusion of the older method, which occurs, for example, in *Oenothera* (Gates, 1907) and has recently been described in detail (Yamaha, 1920) in *Psilotum*. Farr (1916) attempts a physical explanation of the furrowing as a result of the accumulation of electrical charges on surfaces and membranes, and the resulting attractions and repulsions which develop.

The young pollen grains within the mother-cell wall begin at once to alter their shape, each one becoming approximately heptagonal in cross section. This is done while the young pollen grain is surrounded only by a plasmatic membrane, and the change in shape can only be a result of the

cell's own internal activity. It is well known that each genus of higher plants often has its own characteristic form of pollen grain, and it is perfectly certain that the steps towards the assumption of that form which are taken while still within the mother-cell wall must be determined internally by heredity. Whether the later sculpturing of the pollen grain wall is also internally determined, or externally, by apposition of material from the remains of the tapetum, is uncertain. In how far the latter process may take place in the later stages of pollen development has never, we believe, been satisfactorily investigated. But it is clear that the early changes of shape which take place in the young grains within the mother-cell are internally determined in all cases. This was shown, for example, in *Oenothera* (see Gates, 1911, Pl. LXIX, Fig. 47, 48), in which the young pollen grains separate from the centre of the mother-cell, and form within its periphery four discoid cells circular in surface view before the mother-cell wall breaks down. Later the characteristic lobes grow out from three equidistant points in the circular edge of the flattened discoid cells. These are also no doubt internally determined, as is proven, for example, by the fact that while the pollen grains of diploid species have three lobes, those of tetraploid species have four or sometimes more.

By the time the young pollen grains in *Lactuca* have assumed their roughly heptagonal shape, a thin cell membrane has already been secreted around each by the plasmatic membrane of the cytoplasm (Fig. 68). This wall rapidly thickens and develops symmetrical outgrowths (Figs. 70-71) until it reaches the mature stage shown by the grains in Fig. 72. There is a median frill, pinked at the edges, with usually seven, sometimes five, denser radial bands, which are optical sections of other 'frills' running meridionally. In addition, areas of the cell-wall become greatly thickened to form an approximately circular outline. Whether this complicated and nearly symmetrical system of thickenings is laid down by apposition from the tapetal plasmodium we have not determined, but the irregular margin of the outer thickening suggests that this may be the case. Nevertheless, the general arrangement of the thickenings must clearly be controlled from within the cell, and probably the same is true of the circular thickening.

IRREGULARITIES.

Under the heading of irregularities are included (1) an account of pollen mother-cells which are binucleate before, during, or after synapsis, and (2) the occurrence of cytomyxis.

A. Binucleate Pollen Mother-cells.

An interesting feature of the pollen development is the occasional occurrence of binucleate mother-cells. Several such cells are represented in Figs. 14-17. In each of them the two nuclei are in exactly the same

stage of development, and measurements indicate that they are somewhat smaller than the nuclei of uninucleate cells in the corresponding stages of development. The cells, however, are of normal size or only slightly larger. The earliest stage at which binucleate mother-cells were found (and several were seen in this stage) is the presynaptic condition (Fig. 14). They were not traced farther back into the archesporium stage, and the manner of their origin is uncertain. Fig. 15 represents a mother-cell with its two nuclei in synizesis, while Figs. 16 and 17 represent postsynaptic and spireme stages. The latter represents the latest stage in which binucleate mother-cells were found. Theoretically, if such a mother-cell completed the meiotic divisions it should give rise to eight pollen grains, but there is no evidence that this happens. It is possible that such cells break down before the heterotype mitosis. If the meiotic processes were carried through, it is conceivable that pollen grains with two nuclei or a diploid nucleus or some other aberrant condition might result. Possibly some of the aberrant pollen tetrads already mentioned originated from binucleate mother-cells.

Regarding the origin of the binucleate condition in presynaptic cells, we have no absolutely conclusive evidence, but the indications are that it arises through the break-down or incomplete formation of a cell membrane between the two cells.

In the recent literature, a number of cases of binucleate cells in somatic tissue have been described. Their occurrence is so frequent as to indicate that co-ordination between nuclear division and cell-wall formation is by no means universal in growing tissues. The literature of the subject is summarized by Beer and Arber (1920), who have also contributed (1915) many of the recent results. Cells with two or sometimes more nuclei have been observed, particularly in the pith and cortex of the growing region of stems in a variety of plants, such as *Asparagus* (where such cells appear to be particularly frequent), *Eremurus*, *Helianthus*, *Monstera*, *Hemerocallis*, *Elodea*, *Stratiotes*, &c. They found binucleate cells in the growing region of the stem in 177 species belonging to the Pteridophytes, Gymnosperms, and Angiosperms. Polynucleate cells were also found in roots and in the mesophyll of leaves. The binucleate or multinucleate condition was found to arise invariably through mitotic rather than amitotic division. The authors (Beer and Arber, 1919; Arber, 1920) have also shown the manner of origin of the binucleate condition. A cell-wall is not laid down in connexion with the cell-plate of the spindle, but the spindle fibres and associated cytoplasm become transformed into an enlarging hollow sphere which expands until it encloses the daughter nuclei and has been called a phragmosphere. The two nuclei so formed in the same cell may differ somewhat in their later behaviour. One may divide without the other, and this division may be followed by cell-wall formation. In other cases

there are indications that the uninucleate condition may be restored by the degeneration of one of the nuclei.

This binucleate condition in meristematic plant cells is of too frequent occurrence to regard it longer as a mere abnormality. Its chief significance appears to be an indication of an incomplete co-ordination between nuclear division and cell-wall formation. Possibly some unfavourable condition, such as a lowering of temperature at the moment of cell-plate formation, might lead to the development of a binucleate phragmosphere. It appears that the condition is a more or less temporary one, with subsequent restoration of a uninucleate condition in older parts by the stem. There is no indication of a phragmosphere in any of the binucleate mother-cells of lettuce, and, as indicated above, we believe this condition to have originated probably in another way, through the break-down of an incompletely formed cell membrane.

Related phenomena of failure in wall formation after the tetrad divisions have been described in the pollen development of various forms. In *Oenothera gigas* (Gates, 1911, Pl. LXX, Fig. 85), in certain anthers of the flower, the pollen mother-cells failed to round off or free themselves from each other and the tapetum. Such cells not infrequently become quadrinucleate owing to the failure of walls to appear after the reduction divisions, the four nuclei moving together into the centre of the mother-cell. But since these cells are not set free they cannot be functional. Holmgren (1919, p. 13) has described quadrinucleate pollen grains arising in a similar way in *Erigeron erioccephalus* and *E. unalaschkensis* (Fig. 26, p. 14). He finds them only in certain anthers in the flowers which are transitional between hermaphrodite disc-florets and female ray-florets, but believes they might be functional although they develop a thick wall. A fusion of nuclei in such cells might conceivably give rise to pollen grains which were functionally diploid or even tetraploid.

B. *Cytomyxis*.

The condition known as cytomyxis was found to be of relatively frequent occurrence in pollen mother-cells of all species of *Lactuca* under observation (Fig. 13). It is found to occur frequently during synizesis in otherwise perfectly normal mother-cells where the nucleus of one was so eccentric in position as to come in contact with the cell-wall of an adjoining mother-cell. It was not found to occur necessarily in all cells of one locus, nor in the same direction, but merely haphazard according to the position of the nucleus with relation to an adjacent mother-cell.

This condition was found to be even more marked in loculi which showed traces of abnormality, for example those in which all or most of the nuclei appeared hyperchromatic. In extreme cases there was complete transference of the chromatin material from one cell into the cytoplasm of an

adjacent one. Cytomyxis was also observed to take place during the spireme stages, but not so frequently as in synizesis, probably because the mother-cells are more separated at later stages. One case of such transfer of nuclear material was seen at the telophase of the heterotypic division, where it is evidently to be regarded as an abnormality. It seems probable that the phenomenon is not peculiar to any one stage of pollen development, but is merely dependent on the nucleus becoming eccentric and taking up a position where there happens to be an opening at the point of contact with an adjacent cell-wall. That it is in some way connected with a pathological condition of the loculus seems to be indicated by the fact that the phenomenon is of much more frequent occurrence in loculi which are in other ways abnormal than in otherwise perfectly normal ones.

This process, by which a portion of the chromatin from one pollen mother-cell nucleus passes through an opening in the cell-wall into the cytoplasm of an adjacent mother-cell, was first observed by Koernicke (1902) in *Crocus*. The same process was found by Digby in *Galtonia* (1909), *Primula* (1912), and *Crepis* (1914). Gates (1911) described the process in *Oenothera* and called it cytomyxis. Nakao (1911) has seen it in rye. Gregory (1905) observed it in a race of *Lathyrus odoratus* which had sterile anthers, but mistook it for an abnormal division of the pollen mother-cells by constriction. Rosenberg observed it in *Crepis* (1909 a) and also in *Dracera longifolia* (1909 b, Fig. 11, p. 22). Fraser (1914) found it in *Vicia Faba*. West and Lechmere (1915) described it as occurring on a large scale in *Lilium candidum*, although none of the appearances of pollen sterility were found. In this case it must either be normal or induced by the treatment. Sakamura (1916 and 1920) figures it in *Vicia Faba*, and found it much more frequent in anthers which had undergone chloralization than in those which had not.

The most frequent opinion expressed is that the process is an abnormal one, either pathological or induced by the fixation or treatment, and that it will be followed by break-down of the cells in which it occurs. There are, however, difficulties with this view, and in some cases it is followed by return of the nucleus to a central position and absorption of the extruded chromatin in the cytoplasm of the invaded cell.

DEVELOPMENT OF THE TAPETUM.

Some features of the early tapetal cells have already been mentioned (pp. 366, 371). The tapetum is a well-defined layer even at the very early archesporial stages, and is remarkable even then for the elongation of the cells in the direction of the long axis of the anther, that is, at right angles to the usual direction of elongation. The two mitotic divisions of the nuclei in the tapetal cells are usually completed about the end of the synaptic period in the pollen mother-cells. But many of the

tapetal cells remain in the binucleate condition. This is particularly true of cells near the end of a loculus, but one not infrequently finds the tapetal cells quadrinucleate on one side of a loculus and binucleate on the other (Fig. 4). Binucleate cells are nearly always shorter and broader than the quadrinucleate, and in the latter the nuclei are usually, though not invariably, in a single row. As Figs. 1-4 and 73-80 show, there is a great variety of sizes and shapes of cells. Figs. 79 and 85 show two extremes. The long narrow cells are usually towards the centre of the loculus. In these binucleate cells particularly the appearance of the nuclei is extraordinarily like that of some of the synaptic stages in the pollen mother-cell nuclei. Figs. 75-81 show the various appearances of the tapetal cells at about the time of diakinesis. It will be seen that the cells are binucleate or quadrinucleate, with different arrangements of the nuclei and various appearances of the nuclear content. The latter may be in the form of a coarse reticulum with karyosomes at the nodes (Fig. 75), or a finer network with sharply marked chromatin bodies which appear to be more independent of the network (Figs. 77, 78). These bodies are not chromosomes; they are usually rather angular in shape, and are variable in size and number, though often about twelve or thirteen are present. Sometimes they have the appearance of being longitudinally split, and they give the nuclei a hyperchromatic appearance. Winge (1917) has observed and figured (Figs. 5 and 6, p. 150) chromatic bodies having a very similar appearance in the cytoplasm of the young spores of *Entorrhiza Raunkjaeriana*, a member of the Plasmodiophoraceae, at a time when the nucleus is small, amoeboid in shape, and non-chromatic.

Another type of tapetal cell exists in *Lactuca* (Fig. 79), small in size, with small nuclei, having a fine network and only one to four large angular chromatin bodies.

It is difficult to say what proportion of the tapetal cells remain binucleate, but probably it is a considerable number. At a later stage of development the nuclei of the tapetal cells frequently fuse with one another, sometimes forming one large nucleus. Or some of the nuclei may break down (Figs. 81, 85). Finally, at the stage of pollen tetrads the nuclear membranes become very faint (Figs. 83, 86) and disappear, the nuclei more or less completely disintegrating and forming irregular or globular fragments in the cytoplasm. At the same time or a little later the cell-walls, which have become very thin, disappear, and the cytoplasm, containing nuclei in various stages of disintegration, flows in amongst the pollen grains and forms a tapetal plasmodium (Figs. 72, 84).

A tapetal plasmodium was formerly supposed to be characteristic of sporegenesis in Pteridophytes, but not of the Angiosperms. Juell (1915), from a comparative study of the later stages of tapetal development in Angiosperms, finds two extreme types, (1) in which the cells form a true

plasmodium with active nuclei, (2) in which the tapetal cells are emptied of their contents without the breaking down of the cell-walls. Between these extremes every type of intergrade occurs. A plasmodium was found in *Anthurium*, *Lavatera*, *Cobaea*, *Lonicera*, *Valeriana*, and *Knautia*. In *Galium* the tapetal cells form pseudopodium-like incursions between pollen mother-cells. The other type, in which the cell-walls remain intact and the cells are finally resorbed, occurs in *Hyacinthus*, *Galtonia*, *Iris*, *Tilia*, *Ulmus*, *Gaura*, *Sambucus*, *Cucurbita*, &c. In *Arabis* and *Linum* the condition described closely approaches that found in *Lactuca*. The tapetal cell-walls disappear very late, producing in the former a plasmodium with disorganized nuclei and in the latter a plasmodium-like disorganized mass. Tischler (1915) finds that in the Commelinaceae the walls of the tapetal cells are lost during synapsis. The plasmodium then wanders in among the pollen mother-cells, its nuclei undergoing changes in form and structure. It is finally resorbed, so that only a trace remains in the ripe anthers. In *Commelina coelestis* Tischler figures the plasmodium in direct and intimate connexion with the tips of the thickenings on the sculptured walls of the pollen grains.

Pickett (1916) has described in *Arisaema* how the tapetal cell-walls disappear, allowing the periplasm to spread through the sporangial cavity. He says: 'The tapetal nuclei for a considerable period show peculiarities of structure, and take an amoeboid form suggestive of active migration among the developing pollen spore-.' While the type of tapetal history is generally the same throughout a large group, there are exceptions in which the tapetum of related families may show quite different behaviour.

DISCUSSION.

The Method of Synapsis.

From the preceding account it is clear that the method of chrom. some reduction in *Lactuca* is telosynaptic, and essentially in accord with the scheme of Farmer and Moore (1905). There is no indication of a pairing of threads previous to or after synizesis, but the delicate univalent leptone-ma of synizesis gradually condenses into a relatively short and thick pachynema, which arranges itself into as many loops as the gametic number of chromosomes. Clearly the pachynema is a single thick filament composed of the somatic chromosomes arranged tandem, the homologous paternal and maternal chromosomes alternating. The two arms of each loop of the pachynema constitute a pair of homologous chromosomes lying side by side, and owing to torsion these arms or sides of the loop frequently become wrapped around each other. The looped condition apparently corresponds in time with the second contraction phase of other forms. It differs from the bouquet stage, (which apparently occurs in the presence of a centrosome) in

that the loops are radial or irregular in position instead of being polarized, and when the bouquet stage is accompanied by parasynapsis it of course differs also in the constitution of the individual loops.

The complete loops separate from each other at the base in the segmentation of the spireme. Each bivalent chromosome is thus constituted from the two arms of a loop. This structure condenses greatly, and it appears that in some cases at least the torsion remains. If this account of the formation of the bivalents is true, and we can see no escape from it, then synizesis has no part in bringing about the pairing, and its significance as a unique physiological condition of the nucleus remains entirely obscure.

In the later stages of diakinesis the nine bivalents appear chiefly as rods of different lengths, with sometimes a straight line of fission between their two halves. In many cases even at this time the two halves are so closely associated that no line of separation between them can be observed. On the heterotypic spindle these bivalents become still further condensed, but Fig. 58 (Pl. XVIII) clearly shows how in the heterotypic mitosis the longitudinal halves of the bivalents are gradually pulled apart, the separation beginning at one end and proceeding to the other. Whether this line of separation is the same as the line of approximation of the two arms of a postsynaptic loop forming respectively the paternal and maternal members of a bivalent chromosome, depends upon whether the twisting is undone again before the final condensation of the bivalents takes place. In any case this mitosis is essentially a reductional one, although if the separation is across the line of the twists it would only be reductional as regards the individual segments and not as regards whole chromosomes.

This method of reduction differs from that in *Oenothera* (Gates, 1908) only in that in the latter the pachynema segments directly into a chain of chromosomes which are so short and stout that the members of each pair simply come to lie side by side after the chain segments into pairs connected at one end, since there is no opportunity for them to twist around each other. In *Galtonia* (Digby, 1910) and *Primula* (Digby, 1912) the course of events appears to be still more closely similar to that in *Lactuca*. We therefore regard the telosynaptic course of events as clearly established for a number of plants, but we see no necessity for regarding it as universal for the whole Plant Kingdom. Some years ago it was suggested (Gates, 1910) that both the telosynaptic and the parasynaptic methods of synapsis may occur, the latter perhaps more largely in forms with long thready chromosomes and the former in forms with short and stout chromosomes. Ten years ago the senior author had the opportunity of examining critically the beautiful preparations of Janssens (1905) of *Batrachoseps*, and was convinced, as Wilson (1912) and others have been since, that an approximation of filaments or univalent spiremes, accompanied by their polarized arrangement, takes place in this form. Janssens's figures

such as 15 and 36, are particularly convincing, and the original preparations still more so, as showing that the lateral pairing of threads is a progressive process beginning at one end of the threads at one side of the nucleus, and proceeding towards the other end, the double threads so formed becoming at the same time polarized in their arrangement. When this process is partly completed, the filaments may be seen to be lying side by side in the paired portion, but diverging widely in the unpaired portion of their length. This would scarcely be the case if the process were simply one of the approximation of the two halves of a single chromosome.

Agar's (1911) figures of the lepto-zygotene and the zygo-pachytene stages in the spermatogenesis of *Lepidosiren* are equally clear and convincing, for they show the lateral pairing of filaments taking place in precisely the same way. It is probable that the polarization of threads in the bouquet stage is associated with the presence of a centrosome. In higher plants, on the contrary, in the absence of a centrosome, there is no such polarization,¹ and it is possible that this may be associated with the telosynaptic method of pairing.

As the primary purpose of this paper was not a study of synapsis, reference will be made to only a few of the recent papers on this subject. In plants, the most important recent paper is that by Digby (1919) on *Osmunda*. In a very careful investigation of the meiotic and premeiotic divisions in this plant, she emphasizes the fact that the somatic chromosomes in the premeiotic mitoses undergo a split in telophase as a first stage in the process of alveolization, and that in the reverse series of prophase events each chromosome appears to be formed by the re-approximation and fusion of these two split halves or threads. The double thread which every one has observed in the postsynaptic stages of the heterotypic prophase in *Osmunda* is the crux of the matter for the telosynaptic or parasynaptic interpretation. She interprets it as the same doubling which appears in premeiotic prophases, i.e. as the approximation of the two halves of a univalent spireme, and not the pairing of two univalent filaments. Strong and critical evidence is brought in support of this view, but it unfortunately, probably owing to the nature of the material, lacks one crucial fact which would be absolutely determinative: the number of loops or strands before and after this stage has not been determined, and is probably not possible of determination owing to their arrangement.

The Gordian knot of interpretation might be cut, as Hogben (1920) has done, by assuming constant differences between plants and animals in their meiotic processes. But if one compares Miss Digby's figures (1919, Pl. IX, Figs. 47, 48) with Grégoire's (1907) earlier study² of *Osmunda* (Figs. 24, 25) on the one hand, and with Janssens's (1905, Pl. III, Fig. 15) on

¹ The slight indication of polarization of the threads at one transient stage in *Osmunda* may represent an intermediate condition.

Batrachoseps and Agar's (1911, Pl. II, Figs. 12, 13) on *Lepidosiren* on the other, it seems probable that the difference in opinion regarding these forms is entirely one of *interpretation* of this stage. We might be inclined on this basis to conclude for the telosynaptic account in all, but for the fact (1) that the telophase and prophase split in the spireme stage of the somatic chromosomes is by no means universal, and (2) that Hogben (1920), whose figures of these stages in *Periplaneta* are too diagrammatic to be of great value as evidence, nevertheless has the distinct merit of having counted the loops in the bouquet stage in oogenesis and found the diploid number of loops before the pairing and the haploid number afterwards. This appears to show definitely that parasynapsis occurs in *Periplaneta*.

The Orthoptera among animals were long supposed to show telosynapsis, but the latest account in these insects (Wenrich, 1916) gives strong evidence in favour of parasynapsis in *Phrynotettix*. Owing to differences in staining and in form, it was possible to trace one pair of chromosomes, and to a less extent two other pairs, into the late semi-resting telophases of spermatogonial divisions and from the earliest heterotypic prophases through the spireme stages to diakinesis. This method of tracing the history of a single chromosome might be supposed to yield absolutely crucial evidence, but we believe the evidence just falls short of this. Wenrich's Figs. 68, 69 (Pl. VI) show the pair of more deeply staining 'A' chromosomes in the early heterotypic prophase, and like all the prophase chromosomes in *Phrynotettix* he describes them arising, not by the approximation of lateral halves, but as an irregular spiral in a looser matrix. His following Fig. 70 is interpreted as showing the conjugation of the pair of 'A' chromosomes. But this 'pair', which consists of two parallel beaded threads, is apparently thinner than either of the single spiral 'A' chromosomes of the preceding stage. Important and valuable as this paper is, we cannot therefore regard it as a demonstration of parasynapsis, although the evidence as a whole certainly favours that interpretation.

The nature of synizesis and the occurrence of binucleate pollen mother-cells have already been sufficiently discussed in the text.

Chromosome Twisting and Chiasmatty.

The cytological phenomenon described by Janssens (1909) as chiasmatty has been much discussed in recent years as the possible physical basis of the genetical phenomena of crossing-over, particularly in *Drosophila* (Morgan, 1919). As Wilson (1920) has recently pointed out, the original scheme of Janssens involved four strands in a bivalent chromosome, and Morgan (1919) has shown how on the parasynaptic theory with four parallel strands various distributions of the segments of the four chromatids might result. Wilson, who adheres to the parasynaptic method of heterotype chromosome formation in animals, concludes that Janssens's interpretation of

chiasmotypy can only be harmonized with the conclusions of other observers regarding the formation of rings by assuming 'that the chiasmotypy has taken place during a strepsinema stage prior to the straight, longitudinally divided threads from which the rings arise'. He further says (p. 208): 'No observer, so far as I know, has yet seen a process of true crossing-over (recombination) by means of torsion, chiasma formation, and secondary splitting apart.' The torsion described in this paper as occurring during and after the looped stage of the pachynema if followed by breaking across the segments and crossing-over, as appears clearly to be the case in some instances, furnishes exactly the type of redistribution called for, but is obviously quite a different thing from the chiasmotypy of Janssens. This type of twisting is common enough in plant chromosomes during this period. The absence of evidence of such a process in animal chromosomes leads Wilson to suggest that the crossing-over phenomena may find their basis in some process of torsion during or after synapsis. He even suggests some 'internal process of torsion' or rotation in the early pachytene stage before the duality of the diplotene thread becomes externally visible. The clear evidence of torsion in plant chromosomes makes it probable that a similar basis for crossing-over will be found in animals where the phenomenon has been analysed genetically on a large scale.

Chromosome Fusions.

Examination of the literature discloses a considerable number of cases of temporary fusions of various kinds between the chromosomes, both in plants and animals. One of the first cases of a partial or temporary connexion between chromosomes was described by Nawaschin (1912) in root tips of *Galtonia*. Several investigators have shown that in *Galtonia* there are eight pairs of chromosomes of varying lengths. Two of those pairs are very small and lie centrally in the chromosome group. Nawaschin calls them satellites. He finds that in prophase they lie on the surface of the nucleolus. Digby (1910) showed that in anaphase they pass to the poles in advance of the other chromosomes. Nawaschin found one pair constantly attached to the inner end of a certain pair of long chromosomes. Tschernoyarow (1914) has found in *Najas major*, in agreement with Müller (1912), seven pairs of chromosomes which are morphologically distinguishable, one pair being very small. In the heterotypic metaphase Tschernoyarow finds only six pairs of chromosomes, owing to the fusion of these satellites with one of the long pairs. This attachment was also observed in prophase. In this way the discrepancy between Guignard (1899), who counted twelve chromosomes in the reduction divisions, and Müller (1912), who counted fourteen in somatic mitoses, is explained. This type of temporary fusion appears to be similar to the one described in *Lactuca*. It differs, however, in the greater uniformity of its occurrence and in the

presence in other mitoses of a connecting thread between the chromosomes which coalesce in the heterotypic mitosis.

A similar situation exists as regards *Vicia Faba*. Here Fraser and Snell (1911) counted fourteen chromosomes in the sporophyte, and described in some of the chromosomes constrictions which they regarded as somewhat irregular in character. From the work of Sakamura (1920) it appears that one of these constrictions is constantly present in one pair of chromosomes. He figures (Fig. 16) only six bivalents on the heterotypic spindle and concludes that the number of chromosomes should be reckoned as twelve. But Fraser (1914) figures seven bivalents at this time, although two of them appear to be closely in contact. It therefore appears that, as in *Lactuca*, there may be failure of coalescence at this time. Probably the real number here should be considered as twelve, since the large M-chromosome pair shows a median as well as a subterminal constriction. Sakamura (p. 15) finds both these constrictions present in 92 per cent. of cases, but his figures of this and other species of *Vicia* (in some of which he finds twelve and in some fourteen chromosomes) indicate that there is undoubtedly a considerable amount of variation in these constrictions. Again, Fraser and Snell (1911), although they state that seven is the gametophyte number of chromosomes in *V. Faba*, clearly figure six chromosomes in two cases (Figs. 26 and 28) in the first mitosis within the pollen grain, and doubtfully seven in one figure (Fig. 27) of the same mitosis.

These constrictions in *Vicia* and in various other forms (the literature is reviewed by Sakamura) are of a somewhat different nature from the coalescences here described in *Lactuca*. For in the latter there is no indication of connexions in somatic mitoses or heterotypic prophase between the chromosomes which fuse on the heterotypic spindle. The constrictions therefore appear to represent incomplete separation of elements which belong to one chromosome, while the coalescences in *Najas* and *Lactuca* are the coming together temporarily of separate chromosomes. These two series of phenomena are undoubtedly closely related, and *Galtonia* appears to represent a condition intermediate between them. The forms might be arranged in a series, *Vicia*, *Galtonia*, *Najas*, *Lactuca*, with incomplete separation of the parts of a chromosome on the one hand and only temporary coalescence of independent chromosomes on the other.

A phenomenon closely related to the coalescence of bivalent chromosomes in *Lactuca* has been described by Kuwada (1919) in *Zea Mays*, in which the chromosomes are slightly graduated in size. He finds that in starchy varieties of maize the diploid number of chromosomes is twenty, while in certain races of sugar maize the number varies in different individuals. Studies of root-tips in these plants showed 20, 21, or 22, and sometimes 23 or 24. The number of bivalents in the heterotypic mitosis in these forms was 10, 11, or 12. This increase in number is believed to be

the result of transverse segmentation of certain chromosomes. In this connexion it must be mentioned that Collins (1912) on morphological grounds, and as a result of crosses between maize and teosinte (*Euchlaena*), has developed the interesting theory that maize arose as a hybrid between *Euchlaena* and some unknown member of the Andropogoneae. This view is by no means proved and is still being controverted by Weatherwax and others. Kuwada, however, believes he has cytological evidence in its support, for he finds that in hybrids between *Euchlaena* and maize, and also in maize hybrids (Fig. 3, p. 75), some of the bivalents are composed of chromosomes of unequal length. Since the chromosomes of *Euchlaena* (10 pairs) are longer than those of various Andropogoneae, it is thought that the origin of the unequal pairs is to be accounted for in this way. The presence of eleven and twelve bivalents in some individuals of sugar maize is ascribed to a tendency of two chromosome pairs to undergo constriction and transverse segmentation, these two being descended from *Euchlaena* chromosomes. These theoretical conclusions are suggestive, but will require much stronger evidence before they can be accepted.

In animal cytology several clear-cut instances of chromosome fusions are known. Cases in Orthoptera will be briefly considered. McClung (1905) described in three species of *Hesperotettix* a hexad multiple chromosome in the heterotypic prophase formed by the temporary union of a particular tetrad (bivalent chromosome) with the dyad X-chromosome. This union never fails, and there are constant size differences in the hexad of the three species of *Hesperotettix* studied. Such a hexad has been observed in three Orthopteran families, Acrididae, Locustidae, and Phasmidae. In *Hesperotettix speciosus* (McClung, 1917) in the male the X-chromosome becomes attached to a euchromosome before synapsis, forming one limb of a V-shaped element with unequal arms. During synapsis the mate of this euchromosome pairs with it and later separates, but throughout maturation the X remains attached to this chromosome and enters the spermatid nucleus in this condition. In *H. pratensis* the multiple differs from that of *H. speciosus* only in the proportion of its parts, while in the two other species no multiple (i.e. no fusion) occurs. In *H. viridis* the hexad is formed, and also one or two octads may occur by the association of two euchromosome tetrads. The latter process appears to be exactly similar to what occurs in *Lactuca*. The X also may remain free. This gives rise to six types of conditions, in which the apparent number of chromosomes varies from nine to twelve due to different combinations of these fusions. But the particular associations are permanent for the individual, the same type of multiple being found throughout all its germ cells. In the related genus *Mermiria* multiple chromosomes were lacking in two of the species investigated, but in *M. bivittata* a large hexad was formed as in *Hesperotettix*. In all cases

the point of union in metaphase is at the ends of the chromosomes, and the phenomenon appears to differ from that in *Lactuca* only in its greater precision and in its uniformity for the individual.

In the Jamaican Locustidae a similar situation has been described (Woolsey, 1915). In the genus *Jamaicana* the basal number of chromosomes in the males is thirty-five, but some of the chromosomes unite during meiosis in certain species and individuals. The chromosomes form a graded series, the X being the largest. In the spermatogonial metaphase two chromosomes of unequal length unite to form a V while their mates remain separate. This was the condition in seven of the ten individuals belonging to the three species studied. In the three exceptional individuals, one from each species, the conditions were as follows: (1) Two pairs of associated rods forming unequal V's. This gave the appearance of thirty-one chromosomes. These V's separate after the spermatogonial divisions. (2) One large V was formed, each arm being composed of a pair of chromosomes united end to end. (3) The two unequal V's were formed, but in the first maturation division they united to form an elongated ring, the V's separating on the heterotypic spindle. In this genus a multiple is usually accompanied by the X-chromosome. They appear to be attracted, but are not brought into union as in *Hesperotettix*, *Mermiria*, and *Chorthippus*.

The possible relations of these unions of lettuce chromosomes to genetic phenomena as a basis for partial coupling and modification of the Mendelian ratios has been discussed elsewhere (Gates, 1920, p. 221), and need not be considered again at this time.

Chromosome Numbers in Lactuca.

The chromosome counts of Ishikawa (1916) disclose considerable variation in this genus, the x number ranging from five to twenty-four. There are five pairs of small chromosomes in *L. denticulata*, *L. Keiskeana*, *L. lanceolata* and its var. *platyphylla*. In *L. lanceolata* the chromosomes apparently differ conspicuously in size. In *L. stolonifera* Ishikawa finds eight pairs of extremely small chromosomes, and in *L. Tamagawensis* eight, often seven, very small bodies. As all the counts were made during the meiotic divisions, the occurrence of seven bodies in some cases points to a fusion of chromosomes similar to that here described in lettuce. In *L. villosa* and *L. laciniata* there are nine pairs of chromosomes, and they are much larger, apparently agreeing with those of lettuce in their main features, though *L. laciniata* has larger chromosomes than *L. villosa*. In *L. Thunbergiana* the chromosomes are similar in size to those of the last two species, but there are twelve pairs of meiotic chromosomes, or frequently eleven, probably as a result of temporary coalescence of two pairs during meiosis. *L. debilis* is $4x$ as regards chromosome numbers, having twenty-

four pairs, but is probably not tetraploid, since its chromosomes are small and may have been derived through a transverse segmentation rather than a longitudinal split. A critical study of the chromosomes throughout the genus *Lactuca* would be a valuable aid in determining the relationships and phylogeny of its species, and would also throw fresh light on the manner in which chromosome numbers and chromosome morphology change from species to species. Numerous recent papers have furnished preliminary materials for such a study of the phylogeny of chromosomes, which will undoubtedly be one of the important future developments in cytology.

SUMMARY.

In cultivated lettuce (*Lactuca sativa*, L.) there are nine pairs of chromosomes which form a graded series as regards their length. This is true of both the somatic and the meiotic chromosomes. The same number is found in *L. muralis* and probably also in *L. Scariola*.

The method of reduction is telosynaptic, the pachynema forming nine loops, each of which represents a pair of chromosomes attached at one end, the two arms of a loop each constituting a chromosome. These arms frequently twist about each other, before or after the spireme segments into pairs of chromosomes. As these chromosomes condense they may untwist in some cases, but there is some evidence that they frequently break across the twists, producing a straight line of separation between the two longitudinal halves of a bivalent with crossing over of segments from one chromosome to its mate. This differs from the chiasmotypy of Janssens, but furnishes a physical basis for genetical crossing-over.

Synizesis was observed in tapetal nuclei in a few cases. The tapetal cells themselves show several peculiarities. They are loosely arranged even in the presynaptic stages and they show a variety of types, some becoming quadrinucleate and enormously elongated lengthwise of the loculus, others remaining short and thick and often binucleate with very large nuclei. Some of the latter are scarcely distinguishable from pollen mother-cells except by their position. The presence of such transitional forms between pollen mother-cells and tapetal cells probably accounts for the occasional occurrence of synizesis in such cells.

With regard to the nature of synizesis or the synaptic knot, it is found that the process begins with contraction of the nuclear reticulum and is frequently accompanied by the formation of a new precipitation membrane surrounding the reticulum. The contraction is accompanied or followed by a transformation of the reticulum into a spireme, and in the meantime the nuclear membrane undergoes a marked expansion or increase in size. The occurrence of synizesis in tapetal cells emphasizes its unique aspect as a peculiar physiological condition of the nucleus, but since the pairing

of the homologous chromosomes takes place some time later the significance of the contraction remains obscure.

Binucleate pollen mother-cells were found both before, during, and after synapsis. It seems probable that such cells arise by the breaking down of an incomplete wall between two cells in the archesporium stage.

Occasionally in diakinesis only eight chromosome bivalents were present, and frequently there were only seven or eight separate bodies present on the heterotypic spindle. This was found to be due to a temporary end to end fusion of certain bivalents, usually the shorter ones but occasionally the longest being involved. This phenomenon is also likely to disturb Mendelian ratios, causing partial linkage.

The occasional occurrence of ten chromosomes in diakinesis is due to the separation of the members of one bivalent. On the heterotypic spindle the chromosomes are greatly condensed and there is usually no indication of their bivalent character until the halves begin to separate. Constant differences in size can still, however, be observed.

In the heterotypic telophase the chromosomes are already longitudinally split for the homotypic mitosis.

After the reduction divisions, the cytoplasm of the pollen mother-cells begins to constrict at four points, and these constrictions finally meet in the centre, cutting the contents of the cell into four parts. The young pollen grains so formed alter their shape within the mother-cell wall, becoming roughly heptagonal and then secreting a cell-wall. The mother-cell wall then breaks down and the wall of the pollen grain ultimately becomes remarkably thickened and sculptured.

The tapetum forms a plasmodium in which the nuclei are more or less completely disintegrated, the process of disintegration beginning before the break-down of the tapetal cell-walls. The plasmodium flows in among the pollen grains, and may contribute directly to the sculpturing of their walls.

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EXPLANATION OF PLATES XVI-XIX.

Illustrating Dr. Gates and Miss Rees's paper on a Cytological Study of Pollen Development in *Lactuca*.

All the figures were drawn with the camera lucida under a $1/12$ " mm. homog. Kori-ska Ap.N. 1-30 with Comp. Oc. 12 or Comp. Oc. 6, giving magnifications of $\times 3,000$ and $\times 1,500$ respectively.

Figs. 1-17, 55, 56, 64, 70-72, 74, 79, and 82-85 were taken from the rogue.

All other figures from the type.

PLATE XVI.

Fig. 1. Portion of loculus showing pollen mother-cells with nuclei in resting stage. Tapetal cells uninucleate. $\times 1,500$.

Fig. 2. Loculus at later stage. Pollen mother-cells in postsynaptic spireme stage. One mother-cell is binucleate. Tapetal cells with from one to four nuclei. $\times 1,500$.

Fig. 3. Loculus at similar stage to Fig. 2, one tapetal cell showing two nuclei in synizesis. $\times 1,500$.

Fig. 4. Portion of loculus showing variation in shape and size of tapetal cells at opposite sides of loculus. $\times 1,500$.

Fig. 5. Pollen mother-cell with nucleus in resting condition. $\times 3,000$.

Fig. 6. Pollen mother-cell showing the beginning of contraction of the reticulum at one point. $\times 3,000$.

Fig. 7. Slightly later stage than Fig. 6. The nuclear membrane has broken down at one side. First indication of transformation from reticulum to spireme. $\times 3,000$.

Fig. 8. Pollen mother-cell nucleus showing final stage of contraction. Membrane plainly visible, surrounding the reticulum. $\times 3,000$.

Fig. 9. Similar stage to Fig. 8, but membrane less apparent. Reticulum still connected to nuclear membrane by numerous fine threads. $\times 3,000$.

Fig. 10. Similar stage showing both reticular membrane and connecting threads. $\times 3,000$.

Fig. 11. Pollen mother-cell nucleus in typical synizesis stage. $\times 3,000$.

Fig. 12. Pollen mother-cell nucleus showing spireme emerging from the synaptic knot. $\times 3,000$.

Fig. 13. Similar stage to Fig. 12, but showing cytomyxis. Extrusion of chromatic material as taken place from the smaller cell on the left. $\times 3,000$.

Fig. 14. Binucleate pollen mother-cell, both nuclei in pre-synaptic resting stage. $\times 3,000$.

Fig. 15. Binucleate pollen mother-cell. Both nuclei about to form synaptic knot. $\times 3,000$.

Fig. 16. Binucleate pollen mother-cell, nuclei in spireme stage. $\times 3,000$.

Fig. 17. Binucleate pollen mother-cell, probably postsynaptic. $\times 3,000$.

PLATE XVII.

Fig. 18. Early postsynaptic spireme stage, thread nearly continuous, a few free ends visible, nuclear membrane indistinct. $\times 3,000$.

Figs. 19-26. Various stages showing looping of the portions of the spireme to form finally nine bivalent chromosomes. $\times 3,000$.

Fig. 27. Slightly later stage; portions of the spireme, in the form of loops, having broken off. $\times 3,000$.

Figs. 28, 29. Further stages of the segmentation of the spireme. Nine segments can be counted. $\times 3,000$.

Fig. 30. Segmentation completed. Note the four long twisted bivalents and five shorter and more condensed. $\times 3,000$.

Figs. 31-36. Further stages of the progressive condensation of the nine bivalent chromosomes, showing also the torsion. $\times 3,000$.

Fig. 37. Diakinesis with ten chromosomes. The two marked 'a' probably represent the two halves of a single original bivalent chromosome. $\times 3,000$.

Figs. 38, 39. Diakinesis. Nearly all trace of the bivalent nature of the chromosomes is lost. $\times 3,000$.

Fig. 40. Diakinesis. One bivalent chromosome almost split into its component halves. $\times 3,000$.

Figs. 41-44. Diakinesis. Nine bivalent chromosomes. $\times 3,000$.

Fig. 45. Diakinesis. Note threads connecting two pairs of bivalents. $\times 3,000$.

Fig. 46. Diakinesis. Two of the longest bivalents fused end to end. $\times 3,000$.

Figs. 47, 48. Diakinesis. Only eight bivalents in each, no definite evidence of fusion. $\times 3,000$.

Fig. 49. Single bivalent chromosome, showing the loops. $\times 3,000$.

Fig. 50. Heterotypic metaphase in polar view. Only seven independent chromosomes, but those marked 'a' represent fused pairs of bivalents. $\times 3,000$.

Fig. 51. Heterotypic metaphase in polar view. Eight chromosomes, one clearly representing two bivalents fused end to end. $\times 3,000$.

Fig. 52. Metaphase showing nine bivalents. Note points of attachment to spindle fibres. $\times 3,000$.

Figs. 53, 54. Metaphase with eight bivalents. Note again points of attachment, and the ring of dark staining material in Fig. 54. $\times 3,000$.

PLATE XVIII.

Figs. 37 a-55 a. Chromosome bivalents taken from the correspondingly numbered figures in Plate XVII, and arranged in rows to show relative lengths and sizes. $\times 3,000$.

Fig. 55. Metaphase of heterotype division in polar view showing nine bivalents. Note denser surrounding layer of cytoplasm. $\times 3,000$.

Fig. 56. Similar stage in side view. Note again denser area of cytoplasm. $\times 3,000$.

Fig. 57. Early anaphase. Whole chromosomes beginning to separate from each other. $\times 3,000$.

Fig. 58. Later anaphase, showing how the bivalents separate. All but the pairs have split apart. See p. 386. $\times 3,000$.

Fig. 59. Later anaphase, cut. $\times 3,000$.

Fig. 60. Telophase. Nine univalent chromosomes in each daughter nucleus. Most of the chromosomes have already split. $\times 3,000$.

Fig. 61. Abnormal telophase. Nuclei strongly hyperchromatic. Note temporary plate forming in spindle, and also chromatic bodies in cytoplasm. $\times 3,000$.

Fig. 62. Telophase. Chromosomes becoming transformed into a reticulum. Note chromatic bodies in cytoplasm. $\times 3,000$.

Fig. 63. Somatic cell from root tip. Anaphase showing eighteen chromosomes varying greatly in length in each group. Note vacuolate nature of cytoplasm. $\times 3,000$.

Fig. 64. Somatic nucleus in prophase from tissue of young bud, showing chromosomes of various lengths. $\times 3,000$.

PLATE XIX.

Fig. 65. Abnormal pollen mother-cell in heterotypic telophase stage, showing supernumerary nuclei. $\times 3,000$.

Fig. 66. Pollen mother-cell showing tetrad formation. Note invagination of cytoplasm to form the pollen grains. $\times 3,000$.

Fig. 67. Similar stage to above, but the furrows have progressed farther and they cut across the spindle fibres. Fourth grain indicated by dotted lines. $\times 3,000$.

Fig. 68. Tetrad after pollen grains have separated from each other, but are still enclosed in mother-cell. Note heptagonal shape of grains and beginning of thickening of the walls. $\times 1,500$.

Fig. 69. Abnormal tetrad in later stage, after mother-cell wall has disappeared. Note that only three pollen grains are present and central one is binucleate. $\times 3,000$.

Fig. 70. Pollen grain showing thickenings of the wall. $\times 3,000$.

Fig. 71. Pollen grain with still thicker wall and 'frills' in process of formation. $\times 3,000$.

Fig. 72. Portion of loculus with three mature pollen grains. Note sculpturing of grains, and plasmodium formed by breaking down of tapetal cells. $\times 1,500$.

Fig. 73. Small tapetal cell with two nuclei. Pollen mother-cells in diakinesis. $\times 1,500$.

Fig. 74. Tapetal cell, with two nuclei in synizesis. Mother-cells in early spireme stage. $\times 3,000$.

Figs. 75, 77. Binucleate tapetal cells. Mother-cells of loculus in diakinesis. $\times 1,500$.

Fig. 76. Binucleate tapetal cell on larger scale, both nuclei in prophase stage of second division. $\times 3,000$.

Fig. 78. Single nucleus of binucleate tapetal cell. Note chromatic bodies. $\times 3,000$.

Fig. 79. Binucleate tapetal cell from end of loculus. Note only two chromatic bodies to each nucleus. $\times 1,500$.

Fig. 80. Quadrinucleate tapetal cell from middle of loculus. Note length of cell. $\times 1,500$.

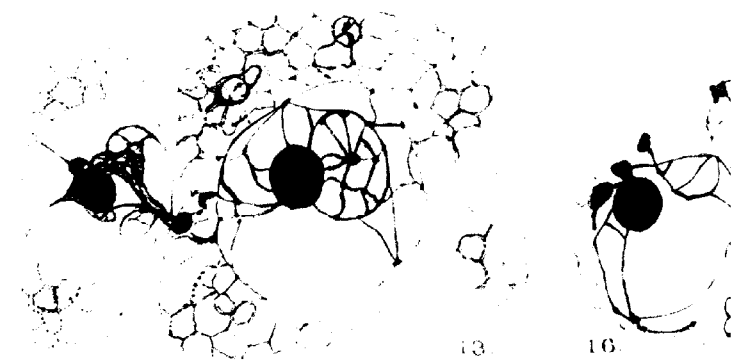
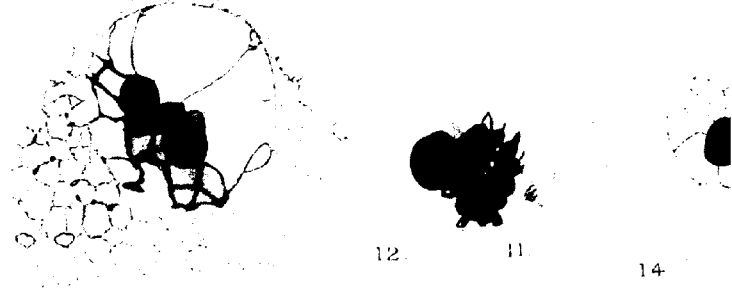
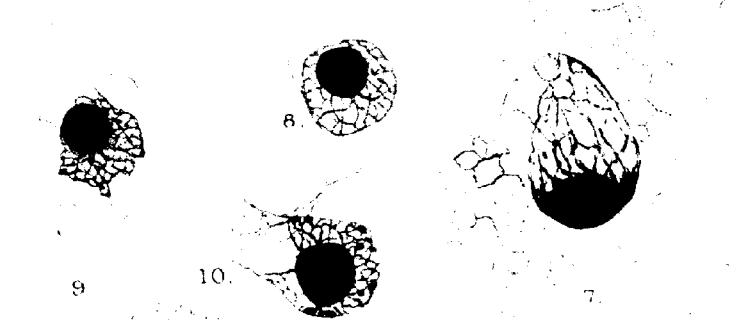
Fig. 81. Quadrinucleate tapetal cell from end of a loculus. Note shape and compare with

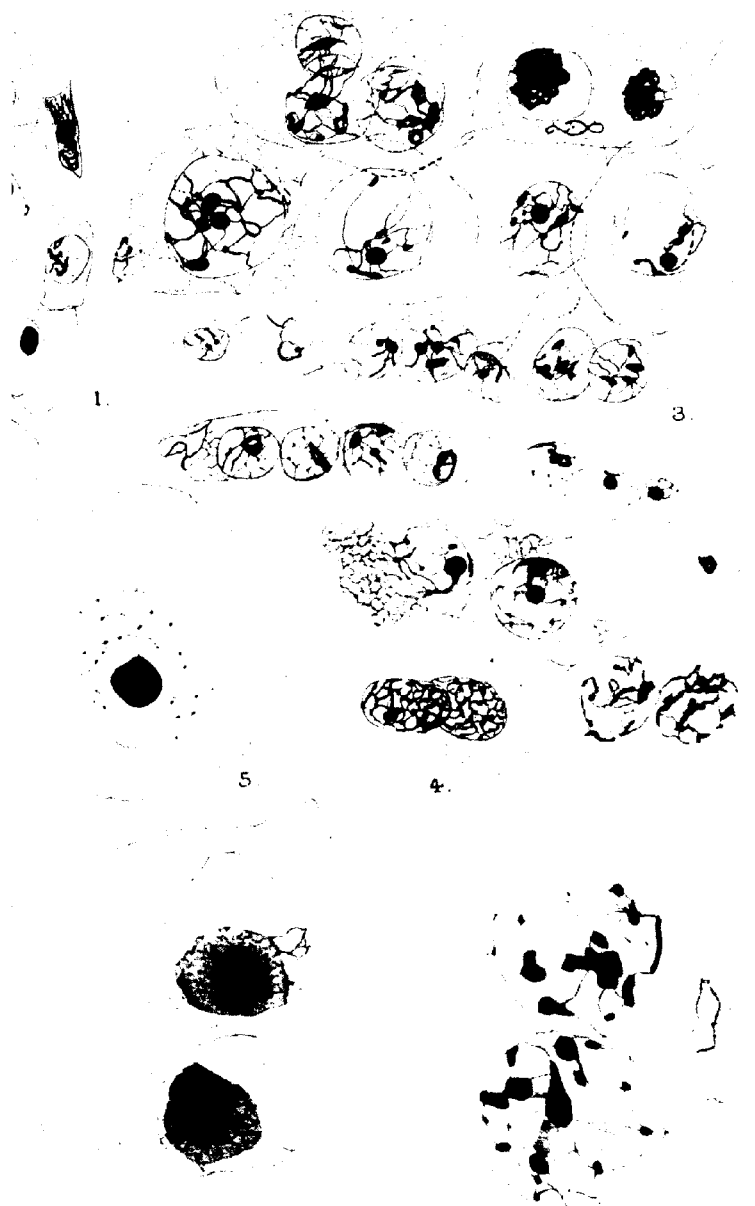
Fig. 80. The two central nuclei are breaking down. $\times 1,500$.

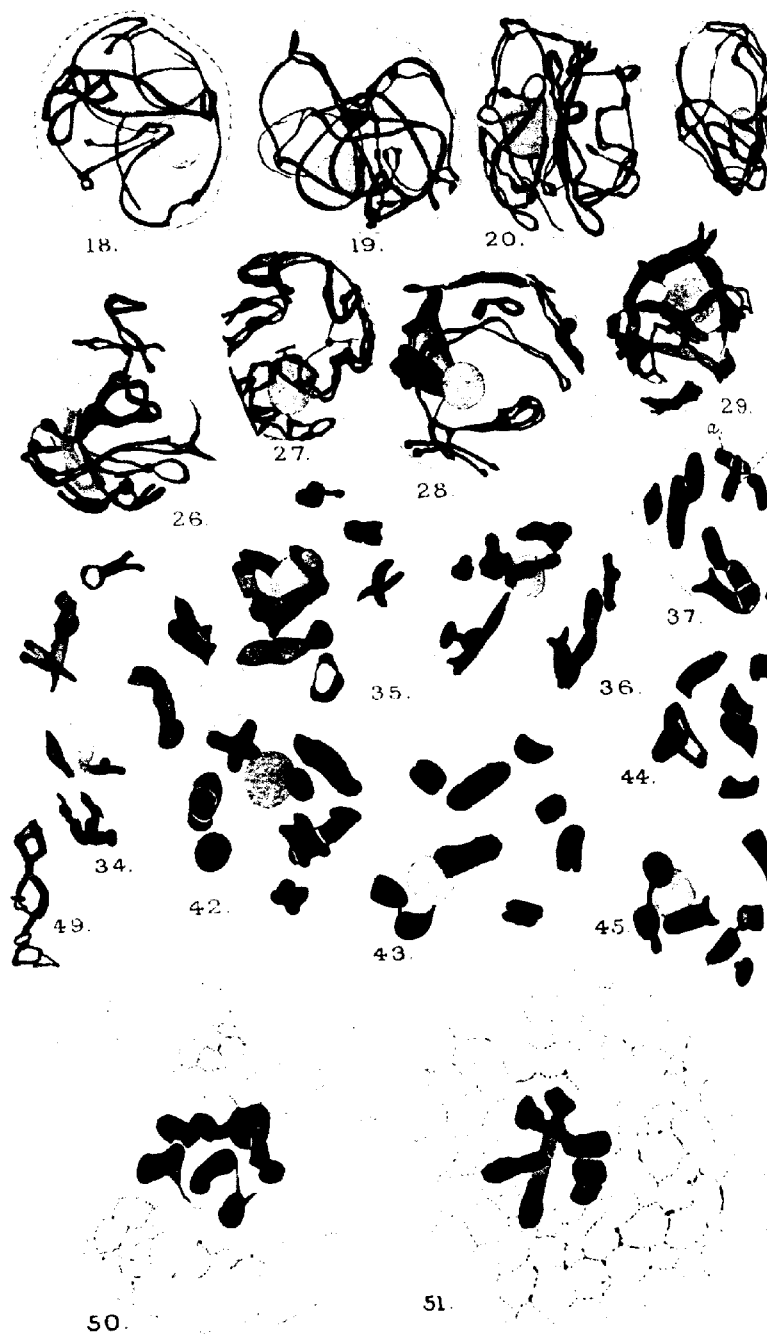
Fig. 82. Trinucleate tapetal cell. Two nuclei in synizesis. Central one apparently result of fusion of two nuclei each in spireme stage. $\times 3,000$.

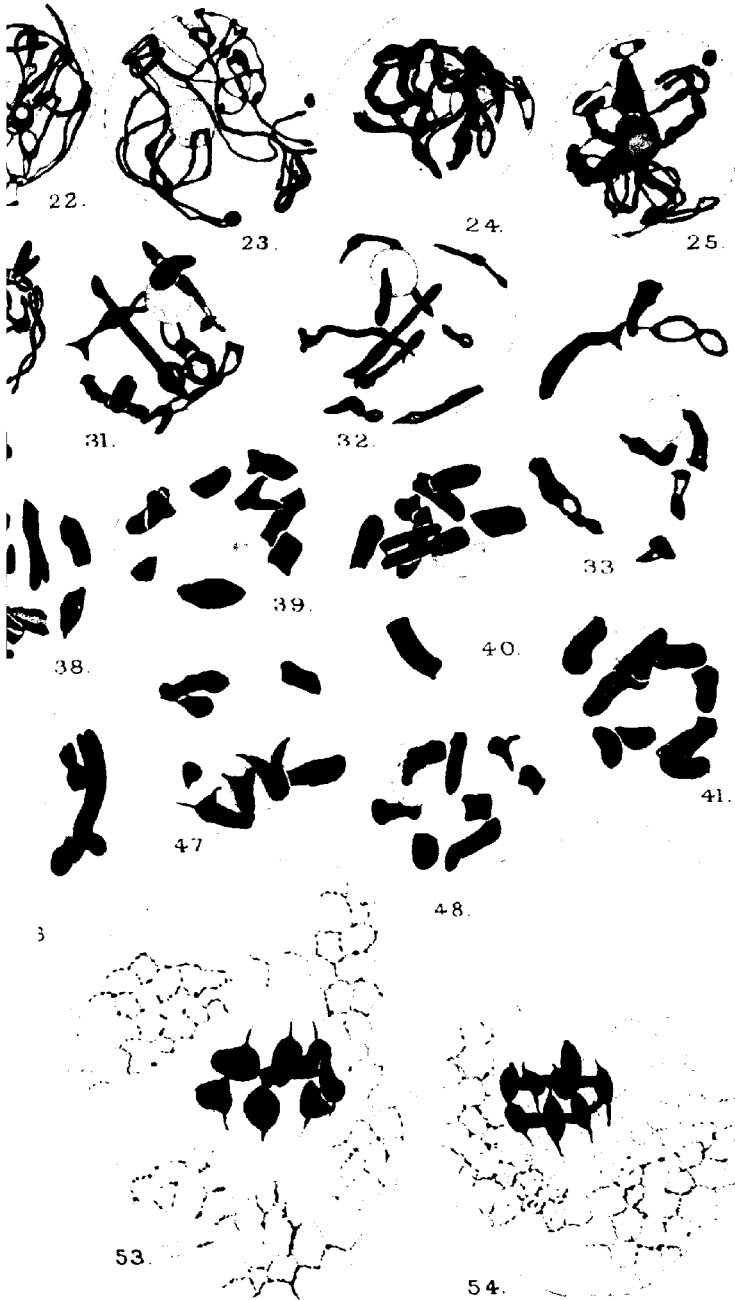
Figs. 83-86. Various stages in degeneration of tapetal cells. Note fragmentation and degeneration of nuclei and disintegration of cell-walls. Mother cells of Figs. 83, 85, and 86 in tetrad stage.

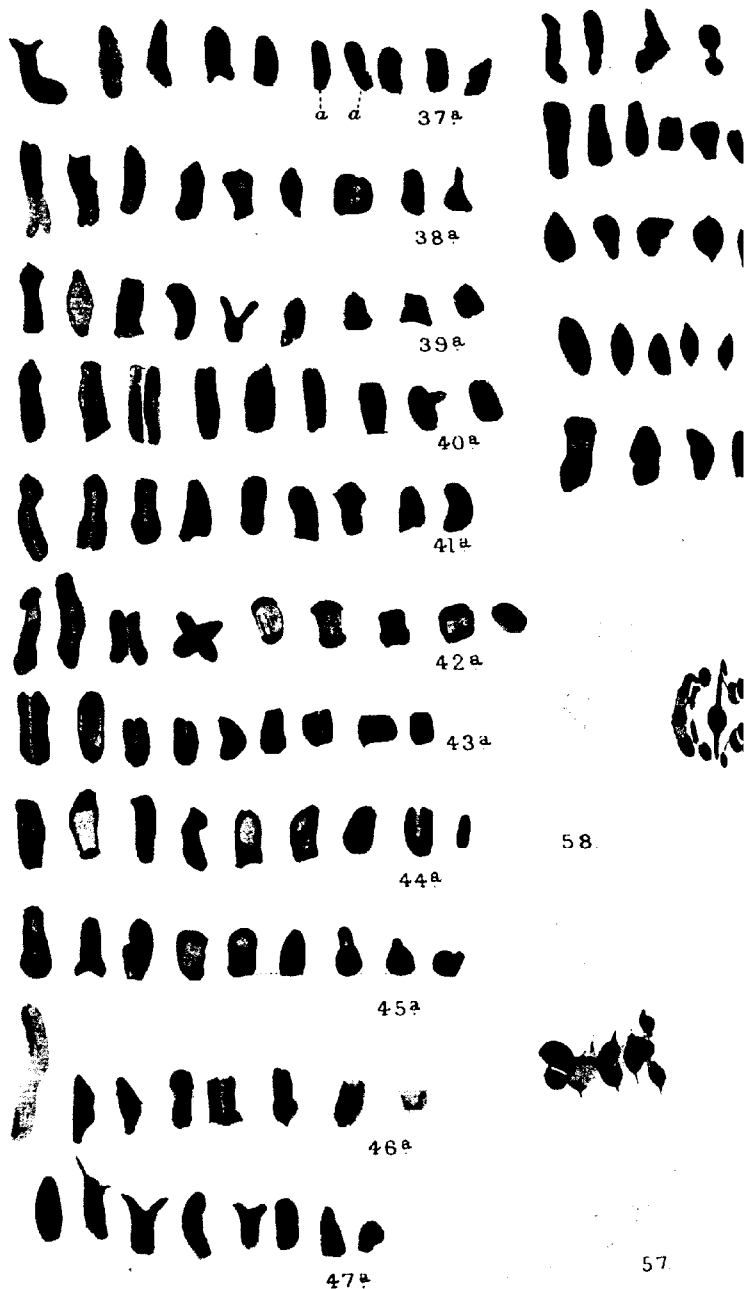
Fig. 84, pollen grains formed. $\times 1,500$.













55.



56.



59



62



64.



61.



63.





Cytology of *Tilletia Tritici*, (Bjerk.) Wint.

BY

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With Plate XX and nine Figures in the Text.

THE investigation which forms the subject of this paper was carried out in the laboratory of Professor V. H. Blackman, to whom my acknowledgements are due for the facilities he has given and for his valuable help and advice.

Bunted wheat seeds required for this work were very kindly supplied to me by Mr. W. L. Waterhouse, of Sydney University, to whom I am much indebted. These seeds were collected in Australia in November, 1918.

My thanks are also due to Dr. S. G. Paine and Mr. R. J. Tabor, of the Royal College of Science, for their many acts of kindness; and to Mr. J. Ramsbottom, of the British Museum, for the facilities he has given me in consulting books from the library.

The cytology of *Tilletia* has been studied in detail by Dangeard¹ (1892), Rawitscher² (1914), and Paravicini³ (1917), and to some extent by Maire⁴ (1902). Dangeard worked on *T. caries*, Tul. (= *T. Tritici*, (Bjerk.) Wint.), Rawitscher on *T. Tritici*, (Bjerk.) Wint., and *T. levis*, Kühn, and Maire and Paravicini on *T. Tritici*.

The spores of *T. Tritici* were germinated on malt extract agar. The germinated spores were fixed in Flemming's weak solution. They were either first evenly spread on a slide smeared with egg albumen, and then the slide was flooded with the fixing solution; or the germinated spores were first fixed in the solution and then minute drops containing the spores were transferred to a slide smeared with egg albumen. The fixing solution coagulates the egg albumen and the spores become firmly held; both these

¹ Dangeard, P. A.: Sur la reproduction sexuelle des Champignons. *Le Bot.*, 3^e ser., 1892, p. 563.

² Rawitscher, F.: Zur Sexualität der Brandpilze: *Tilletia Tritici*. *Ver. d. Deut. Bot. Ges.*, vol. xxxii, 1914, p. 310.

³ Paravicini, E.: Untersuchungen der Zellkerne bei Fortpflanzung der Brandpilze. *Ann. Myc.*, vol. xv, 1917, p. 75.

⁴ Maire, R.: Sur la coexistence de la nielle et de la carie dans les grains de blé. *Bull. Soc. Myc. Fr.*, vol. xviii, 1902, p. 132.

methods give equally good results. The spores were treated with the fixing solution for thirty minutes or more. The preparations were bleached with hydrogen peroxide and stained with Heidenhain's haematoxylin. Breinl stain and the combination of gentian-violet and orange G were also used to check the results obtained with haematoxylin.

The spore germinates by rupturing its wall and produces, as a rule, one promycelium. At times two or three germ-tubes or promycelia are put forth, but all except one degenerate. As in *T. caries*,¹ the nucleus of the spore passes undivided into the promycelium. The migration of the nucleus into the promycelium does not necessarily take place immediately after the promycelium is developed; at times it is long delayed, so that there may be a long promycelium without a nucleus. As observed by Dangeard, the first division of the nucleus may take place soon after it enters the promycelium (Pl. XX, Fig. 2), or the division may be delayed for a time (Pl. XX, Fig. 4), and may be even subsequent to sporidia formation (Pl. XX, Fig. 1). Rawitscher² states that the first division, which he thinks may perhaps be the reduction division, takes place in the spore; it is evident that he merely assumes this, because he has been not only unable to see the first division on account of the opaque wall of the spore, but he has not even observed the later divisions as a result of which eight nuclei are found in the promycelium. He says: 'Die ersten Teilungen desselben—wahrscheinlich die Reduktionsteilungen—liessen sich indes bisher noch nicht beobachten, da sie in den untersuchten Fällen bereits in der undurchsichtigen Spore vor sich gingen. Sobald aber deren ganzer Inhalt in den Keimschlauch eingewandert ist, lassen sich darin zunächst stets acht Kerne zählen.' Paravicini³ also states that the nucleus of the spore divides at the time of germination and that one of the two nuclei passes into the promycelium. The same author has further shown that with the division of this daughter nucleus the tip of the promycelium becomes bifurcated and a nucleus enters each of these branches. The nuclei again divide twice successively and thus eight nuclei are formed; at the same time eight small projections are developed at the tip of the promycelium, and each of these small projections bears what he calls an 'endconidium', each of which is provided with one nucleus. Neither Dangeard, Rawitscher, nor the writer has observed the division of the nucleus to be associated with the threefold bifurcation of the tip of the promycelium and the presence of the nuclei in the very young sporidia. The divisions of the original nucleus do not take place at any particular stage in the development of the promycelium. As in the case of the first division the ultimate divisions may be delayed till the formation of the sporidia. Thus there may be eight nuclei in a promycelium which shows as yet no trace of sporidial formation (Pl. XX, Fig. 5); and there may

¹ Dangeard, P. A.: loc. cit., p. 265.

² Rawitscher, F.: loc. cit., p. 312.

³ Paravicini, E.: loc. cit., p. 76.

be a less number of nuclei in a promycelium which already bears full-sized sporidia (Pl. XX, Fig. 1), or which shows evident signs of sporidial formation (Pl. XX, Figs. 6 and 7). The forking or bifurcation of the promycelial tip described by Paravicini has been observed, but in no case has it been found to be associated with nuclear divisions (Pl. XX, Figs. 1, 6, 7, and 9). Paravicini finds the sporidia nucleated, even when they are very young (cf. his Pl. V, Fig. 5). But Rawitscher¹ finds eight nuclei in the promycelium after it has grown to some length; and his figure distinctly shows the young sporidia to be without nuclei, though there are four pairs in the promycelium. Dangeard² explicitly states: 'Les sporidies dans le *Tilletia* sont aciculaires; au début, leur protoplasme est très dense, homogène; à ce stade, il n'y a pas encore de noyau; plus tard, il devient vacuolaire...; c'est le moment où les noyaux se distribuent dans les sporidies.' The present observations on this point are in complete accord with those of Dangeard. As long as the sporidia are not vacuolated the nuclei remain in the promycelium (Pl. XX, Figs. 8 and 9).

Paravicini³ finds the promycelium to be one-celled, or at the most two-celled, and in the latter case both these cells are nucleated. This observation is again at variance with those of Dangeard,⁴ Rawitscher,⁵ and the writer. The promycelium has been observed to be only one-celled. At times the protoplasm collects in the upper part of the promycelium, and then the empty lower part is cut off by one or more septa; this generally happens in the case of a long promycelium (Pl. XX, Fig. 6).

Dangeard limits the number of nuclei to eight, but as observed by Rawitscher and Paravicini there are, at times, more than eight nuclei in the promycelium (Pl. XX, Figs. 9, 11, and 12). Dangeard states that if the promycelium branches the eight nuclei pass into the new branch; but this has not been found to be invariably the case (Pl. XX, Fig. 12).

The division of the nuclei in the promycelium does not necessarily take place simultaneously (Pl. XX, Figs. 3, 5, 7, and 11). As suspected by Dangeard, the nuclear division appears to be mitotic (Pl. XX, Fig. 3).

The primary sporidia are, as a rule, borne apically, but on rare occasions they are developed on a lateral branch (Pl. XX, Fig. 10). They are usually carried on short forked branches of the promycelium (Pl. XX, Figs. 1 and 9). As already pointed out, Paravicini explicitly states that on the tip of the promycelium eight protuberances are developed, each of which bears an 'endconidium' or sporidium; but in his Fig. 6 of Plate V the sporidia are shown to be borne directly on the flat promycelial end. The number of branches has not been observed to correspond to the number of sporidia.

¹ Rawitscher, F.: *loc. cit.*, p. 313.

Paravicini, E.: *loc. cit.*, p. 76.

³ Rawitscher, F.: *loc. cit.*, p. 312.

² Dangeard, P. A.: *loc. cit.*, p. 266.

⁴ Dangeard, P. A.: *loc. cit.*, p. 265.

⁵ Dangeard, P. A.: *loc. cit.*, p. 265.

The sporidia are at first in direct continuity with the promycelium (Pl. XX, Figs. 1, 8, and 9); the sporidium is cut off from the promycelium by a septum only after the nucleus has passed into the sporidium* (Pl. XX, Fig. 27). The contents of the sporidia are at first dense, granular, and homogeneous; at this stage the sporidia are invariably without nuclei, but later they become vacuolated, and only then does the migration of the nuclei from the promycelium take place.

The sporidia are generally eight, but it is not unusual to find a larger or smaller number; four to sixteen have been observed. It sometimes happens that the promycelium has more nuclei than sporidia, or more sporidia than nuclei; what is the ultimate fate of the surplus nuclei and sporidia is not known. The sporidia are not always uninucleate; occasionally they are binucleate (Pl. XX, Fig. 17), but more than two nuclei have never been observed. In Fig. 39 of Plate XX the right-hand sporidium has two nuclei. These two nuclei may have passed into the sporidium directly from the promycelium, or the nucleus from the central sporidium may have crossed over into the other sporidium through its unseptate base.

Dangeard¹ has observed that the nucleus, during its migration into the sporidium, is at first elongate till it reaches the middle of the sporidium, when it becomes globular. But it is not unusual to find an elongated nucleus in the middle of the sporidium and a rounded one at the base. As a rule the nucleus is found in the lower half of the unconjugated sporidium, seldom in the upper half. Whether this has any physiological significance it is difficult to say.

The conjugation of the sporidia takes place when they are still attached to the promycelium or after they fall off. As a rule the sporidia are linked by means of a single short conjugating tube; but at times a pair are joined by two such tubes. The conjugation in most cases is between two sporidia, but on rare occasions there is a triple conjugation between the sporidia; what happens to the third nucleus has not been observed. The nuclei of the conjugated pair proceed towards the connecting bridge and then the nucleus from one sporidium passes into the other (Pl. XX, Fig. 13). It is interesting that the nuclei in both the sporidia are generally at the same level in the two cells as they migrate towards the bridge (Pl. XX, Fig. 14). As the nucleus passes through the bridge it becomes elongated. Not only is there a passage of the nucleus from one sporidium into the other, but of the whole or part of the cytoplasm as well. After the cytoplasm has withdrawn from the upper and lower ends of the sporidium, septa appear at the free surfaces; the empty or half-empty sporidium ultimately shrivels up. From the sporidium with the two nuclei and extra cytoplasm a germ-tube is given out, at the top of which a secondary sporidium is developed. It very easily falls off from its stalk. It can be readily distinguished from the

¹ Dangeard, P. A.: *loc. cit.*, p. 266.

primary sporidium by its falcate shape and size; it is shorter and broader than the primary sporidium. In the majority of cases observed the secondary sporidium has been found to be uninucleate (Pl. XX, Figs. 18, 24-7, 33, and 38). Dangeard, Rawitscher, and Paravicini have always found the secondary sporidium to be binucleate. Paravicini,¹ however, states that since the two nuclei in the 'Sichelkonidien' (i.e. the secondary conidia) are situated equatorially, they can be distinguished only with difficulty. It is probable that he may have really seen the uninucleate 'Sichelkonidien'.

There seems to be no doubt that the majority of secondary conidia observed in the present investigations are really uninucleate. This point has been confirmed many a time by the use of Heidenhain's haematoxylin, Breinl stain, and gentian violet-orange G combination. This point has also been confirmed in the case of *Tilletia* on wheat collected by my friend Mr. W. L. Waterhouse from Pembroke, Wales, in February. Where and when the fusion between the two nuclei of the conjugated sporidia takes place it is difficult to decide. However, a few distinct cases of the fusion in the secondary sporidium have been found (Pl. XX, Fig. 30). The secondary sporidium may bud off a tertiary sporidium (Pl. XX, Fig. 19), or, what is more commonly the case, it puts forth a germ-tube which may develop a tertiary sporidium (Pl. XX, Figs. 32, 34, and 37). The tertiary sporidium is usually smaller than the secondary sporidium, and always uninucleate. The secondary sporidium may become septate on germination, like the primary sporidium (Pl. XX, Figs. 34 and 37).

The secondary sporidia, in some cases, show definitely two nuclei (Pl. XX, Figs. 22, 28, 29, 30, and 34). At times the two nuclei are so close together as to arouse the suspicion that the pair is in the process of fusion (Pl. XX, Fig. 23). The nucleus of the secondary sporidium passes as a whole into the germ-tube and the tertiary sporidium (Pl. XX, Figs. 32 and 33), or it divides either before or after the germination of the sporidium (Pl. XX, Figs. 21, 35, and 36). In a few cases the division has been found to have begun while the secondary sporidium is still attached to the germ-tube of the primary conjugated sporidium (Pl. XX, Fig. 21). One of the daughter nuclei passes into the germ-tube or the tertiary sporidium and the other remains in the sporidium (Pl. XX, Fig. 19), possibly to provide for further germ-tube or sporidial formation. This division may give rise, at times, to a temporary binucleate condition, either on account of the early division of the nucleus before the secondary sporidium has germinated or delayed migration of one of the daughter nuclei into the germ-tube. And therefore it is difficult, if not impossible, to determine the pedigree of the two nuclei found in the sporidium—whether they are the original two conjugate nuclei or whether they are the daughter nuclei of the single nucleus which has resulted from the fusion of the two conjugate nuclei.

¹ Paravicini, E.: loc. cit., p. 76.

The nuclear condition of the hyphae in the very early stages of infection of wheat seedlings from two to eight days old has also been studied. Seedlings a day old were inoculated with cultures from germinated spores, and then were incubated at 50° C., and fixed in Flemming's weak solution from time to time.

The infecting hypha, as a rule, enters the first or outermost leaf sheath between the epidermal cells. It makes its way in by pushing aside the adjoining walls of these cells (Text-figs. 1-7), and at times the walls are consequently ruptured (Text-fig. 7). Direct entry of the germ-tube in the lumen of the epidermal cells takes place very rarely (Text-fig. 8).

The infecting hypha and the hyphae of the later-formed mycelium are uninucleate or multinucleate (Text-figs. 1-9); in no case were the hyphae uniformly binucleate. In some cases two nuclei in close association were observed, but they may be the daughter nuclei of a recently divided nucleus, or the paired condition may be accidental and temporary.

Schmitz,¹ in 1879, investigated *Ustilago longissima*, (Sow.) Tul., and found the hyphae from which the spores developed to be multinucleate. According to Fisch² the hyphae of *Tilletia*, *Urocystis*, and *Ustilago* are mostly multinucleate. Dangeard,³ as the result of his researches in the cytology of several members (including *Tilletia*) of Ustilagineae, states that the mycelium of this group is multinucleate. But Lutman,⁴ disagreeing with these authors, says his observations show that 'this statement that the mycelium is made up of multinucleated cells is probably true of the genus *Ustilago*, but it is not true of the Tilletiaceae'. He believes the Tilletiaceae to be binucleate; and Maire,⁵ Rawitscher,⁶ and Paravicini⁷ also agree that in *Tilletia Triticæ* the hyphae are binucleate.

The observations described in this paper, as far as they concern the nuclear condition of the hyphae in the early stages of infection, support the views of Fisch and Dangeard.

Rawitscher and Paravicini agree that the binucleate stage in the life-cycle of *Tilletia* is the result of the conjugation of the primary sporidia, and that the binucleate condition continues up to the development of spores, in which the two nuclei fuse, with the consequence that the mature spore is uninucleate. Here it may be noted that there seems to be some doubt as to the correctness of Paravicini's observations of the binucleate condition of the *Tilletia* mycelium in the host plant, because the mode of spore formation

¹ Schmitz, F.: Untersuchungen über die Zellkerne der Thallophyten. Verh. des naturhist. Vereins der preuss. Rheinlande u. Westfalens, 1879, p. 361.

² Fisch, C.: Ueber das Verhalten der Zellkerne in fusionirenden Pilzzellen. Bot. Centrallbl. vol. xxiv, 1885, pp. 221-222.

³ Dangeard, P. A.: loc. cit., p. 269.

⁴ Lutman, R. F.: Life-History and Cytology of the Smuts. Wisc. Acad. Sc. Arts and Letters, vol. xvi, Pt. II, No. 4, 1910, p. 1219.

⁵ Maire, R.: loc. cit.

⁶ Rawitscher, F.: loc. cit., p. 313.

⁷ Paravicini, E.: loc. cit., p. 77.

he has described is quite unlike that generally occurring in the *Tilletiaceae*. The young angular spores he has figured are very similar to those of an *Ustilago*, and he finds that the spores are formed inside a hypha; it seems possible that this 'hypha' is the mucilaginous sheath round the immature spores, which is so characteristic of *Ustilago*.



TEXT-FIGS. 1-7. Penetration of the infecting hyphae through the epidermal intercellular spaces. The hyphae have one or more nuclei. $\times 1,200$. Fig. 8. Penetration of the infecting hypha directly through the lumen of the epidermal cell. $\times 1,200$. Fig. 9. Multinucleate mycelium in the tissues of the leaf-sheath. $\times 1,200$.

The observations recorded in the present investigations show that the binucleate condition is of very short duration, the secondary sporidium being as a rule uninucleate. This difference between these observations and those of the previous workers is, however, not fundamental, but only one

of degree. The important points in the life-history are the fusion of the two nuclei and the uninucleate nature of the mature spore. It is of secondary importance at what stage the fusion takes place; it may be delayed until the development of the spore, or it may take place soon after the conjugation of the sporidia. In the Ustilagineae and in other Basidiomycetes there is indefiniteness as to the place of origin of the association of the nuclear pairs. Federley¹ finds in *Ustilago Tragopogi pratensis*, Pers., the fusion of nuclei taking place immediately after conjugation, and therefore he has not observed the binucleate stage. In *U. Carbo* the binucleate condition plays a very conspicuous part in the life-history; but in *U. maydis* the uninucleate stage is predominant, according to Rawitscher.² Werth and Ludwigs³ have found even the youngest spore of *U. anthrarum*, Fr., to be uninucleate, but they do not say when the fusion takes place.

SUMMARY.

The cytology of *Tilletia Tritici* has been studied.

The spore, on germination, produces a single-celled sporidium.

The nucleus from the spore passes undivided into the promycelium.

The divisions of the nucleus do not take place at any fixed stage in the development of the promycelium.

The divisions of all the daughter nuclei do not necessarily commence simultaneously.

The number of nuclei is variable, but generally eight.

The sporidia are usually eight, but they may vary from four to sixteen.

The migration of the nuclei into the sporidia is delayed till the latter become vacuolated.

Each sporidium has usually a single nucleus and never more than two.

The conjugation of the sporidia takes place either when they are still attached to the promycelium or after they fall off.

As a result of the conjugation the nucleus and part or the whole of the cytoplasm of one sporidium pass into the other, which then germinates and develops a secondary sporidium.

The secondary sporidium is small and sickle-shaped; it is generally uninucleate, but at times is binucleate.

In a few cases the fusion of the conjugate nuclei has been observed in the secondary sporidium.

¹ Federley, H.: Die Copulation der Conidien bei *Ustilago Tragopogi pratensis*. Öfvers. Finsk. Vetensk. Soc. Förhandl., vol. xlvi, 1903-1904, p. 12.

² Rawitscher, F.: Beiträge zur Kenntnis der Ustilagineen. Zeitschr. Bot., vol. iv, 1912, p. 703.

³ Werth, E., and Ludwigs, K.: Zur Sporenbildung bei Rost- und Brandpilzen. Ber. der Deutsch. Bot. Ges., vol. xxx, 1912, p. 224.

The presence of the two nuclei in the secondary sporidium is, at least in some cases, due to the division of a single non-conjugate nucleus.

The secondary sporidia germinate and may develop tertiary sporidia which are uninucleate.

The hyphae, at least in the early stages of the infection of wheat seedlings, are uninucleate or multinucleate.

BOTANICAL LABORATORY OF THE IMPERIAL COLLEGE
OF SCIENCE AND TECHNOLOGY.
September, 1920.

EXPLANATION OF PLATE XX.

Illustrating Mr. Jehangir Fardunji Dastur's paper on the Cytology of *Tilletia Tritici*, (Bjerk.) Wint.

Fig. 1. A germinated spore; the promycelium bears nine sporidia; it has only two nuclei. $\times 1,300$.

Figs. 2-5. Germinating spores. In Fig. 2 the promycelium has a dividing nucleus. In Fig. 3 the lower of the two nuclei in the promycelium is dividing. In Fig. 5 the lowermost nucleus in the promycelium is dividing. Figs. 2 and 3, $\times 1,450$. Figs. 4 and 5, $\times 1,300$.

Figs. 6 and 7. Early stages in the development of the sporidia. Fig. 6, $\times 1,100$. Fig. 7, $\times 1,300$.

Figs. 8 and 9. Sporidia fully developed; the nuclei are still in the promycelium. In Fig. 9 two nuclei are seen migrating towards the sporidia. $\times 1,300$.

Fig. 10. Sporidia on a lateral branch. $\times 1,750$.

Figs. 11 and 12. Branched promycelia with several nuclei, some of which are seen dividing in Fig. 11. Fig. 11, $\times 1,300$. Fig. 12, $\times 1,100$.

Figs. 13 and 14. Conjugating sporidia. $\times 1,300$.

Figs. 15 and 16. Uninucleate sporidia. $\times 1,450$.

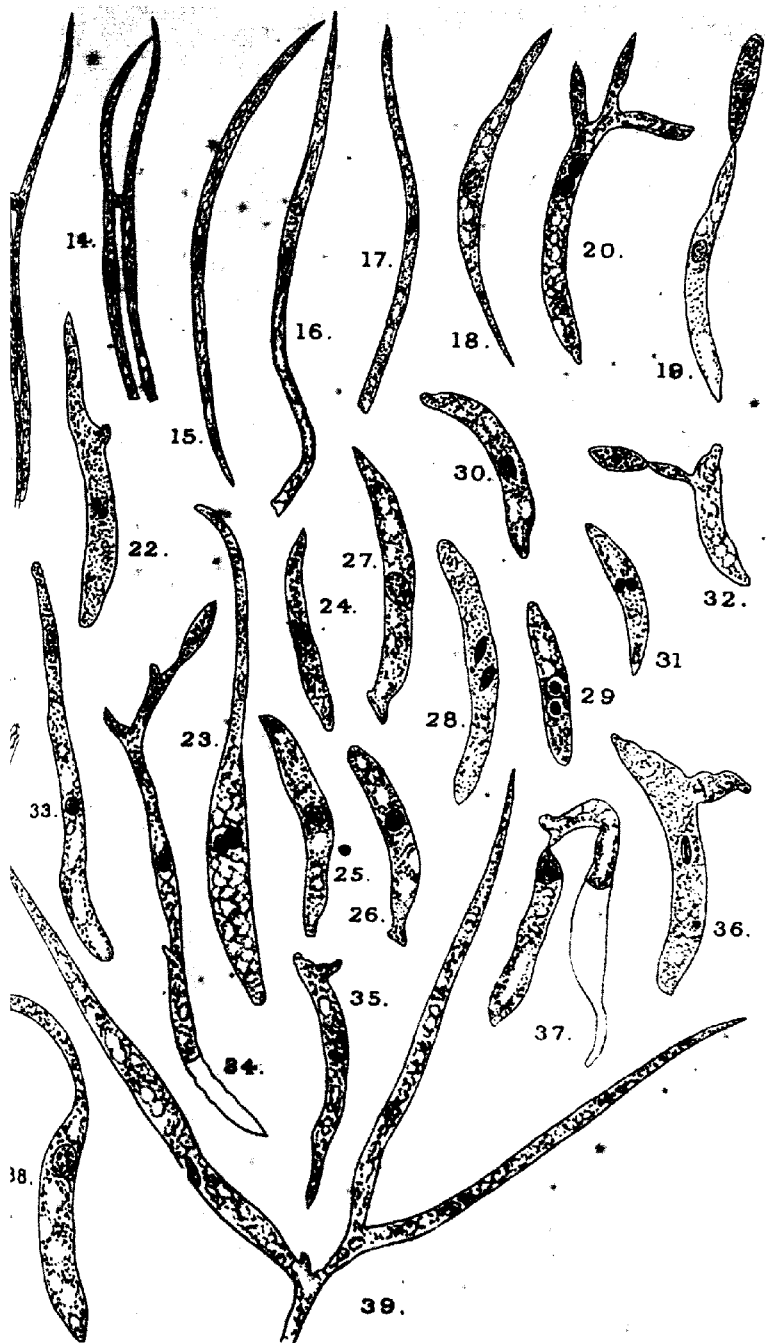
Fig. 17. A binucleate sporidium. $\times 1,300$.

Figs. 18-38. Secondary sporidia, some of which are germinating. In Fig. 21 the secondary sporidium is seen attached to a short germ-tube, which has developed from a conjugated sporidium. The nucleus is dividing. In Fig. 18 the nuclei are fusing. In Figs. 23 and 24 the nuclei are dividing. Figs. 18-21, 23, 24, and 26, $\times 1,450$. Figs. 22 and 25, $\times 1,300$.

Fig. 39. A group of three sporidia. $\times 1,300$.



LE DASTUR - TRITETIA TRITICI.



Observations on Variations in the Flowers of *Stachys sylvatica*, Linn.

BY

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With five Figures in the Text.

FOR some years the writer has observed, in the Hedge Woundwort, the presence of flowers varying, more or less markedly, from the normal. At first notes and sketches only were made of typical variants and also of the more striking ones; but in 1919, for a short time, in the early autumn, a preliminary statistical study of these forms was begun. It should be stated that the variations recorded have been found on sandy, chalky, and loamy soils, in different localities (e.g. at Haslemere in Surrey, Shoreham in Kent, and in the neighbourhood of High Wycombe in Buckinghamshire) and at different times of the year, namely, from June to October. The observations so far made, however, do not enable one to judge how far the soil and the time of year influence either the percentage or the nature of the variations.

TABLE I. SERIES A. *Haslemere. August, 1916.*

	K.	C.	A.	G. [*]	N.	Remarks.	Fig. 1.
1. f.	8	2+3	6	...	10	M.F.	d
2. f.	5	3+3	5	2	...	M.F., 1 N.F. on each side	a
3. f.	5	3+3	5	5	...	M.F., 1 N.F. on each side	e
4.	5	2+3	5	5
5.	5	2+3	4	2
6.	5	1+3	7	2	4	M.F.	...
7.	4	2+3	5	3	5	M.F.	...
8.	5	2+3	5	2	4	M.F.	...
9.	5	1+3	4	2
10.	5	2+3	5	2
11.	5	2+3	4	2	* 4	M.F.	...

f. = fusion. M.F. = middle flower. N.F. = normal flower.

The first series (see Table I) that was observed was collected at Haslemere in August, 1916. The tendencies here shown were towards an increased number of parts leading up to cases of fasciation (Fig. 1, a, d, and e). A large number of flowers were found in which the upper lip of the corolla was larger and wider than usual and a smaller number in which it

was distinctly notched at the apex (Fig. 1, *f*). Of these, unfortunately, no record was made. There were other forms, however, which were provided with five stamens instead of the usual four; some of these showed three stigmas, two had five 'nutlets' (Fig. 1, *c*) recorded for them (records were seldom made of the number of nutlets), most of them had notched upper lips to the corolla, and two of them had two upper lips and in each case one of these lips was notched. These two cases should probably be regarded as incipient fasciations. One of the specimens was remarkable in that it possessed only four sepals, while the other members of all the other whorls were increased (Table I, 7). In the other cases an increase

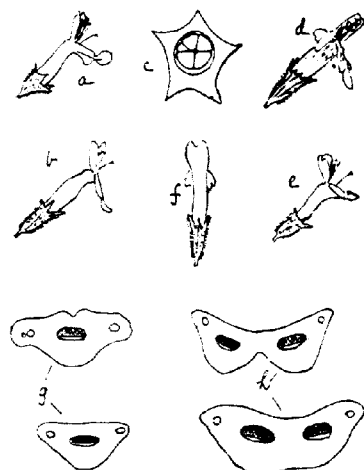


FIG. 1. (See Table I.) Semidiagrammatic. *a*, *b*, *d*, *e*, fasciated flowers; *f*, bifurcated upper lip; *c*, five-lobed ovary seen from above; *g*, sections through normal petiole; *h*, sections through abnormal petiole. [Haslemere, Surrey, 1916.]

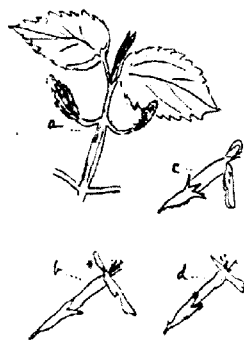


FIG. 2. (See Table II.) *a*, two shoots partly fused to main stem. For description see text and table. [Downley, near High Wycombe, Bucks, 1917.]

in the number of members of any one whorl was usually accompanied by an increase in some other whorl. The undoubted fasciation referred to had eight members to its calyx, two upper corolla lips both bifurcated, six stamens, and ten nutlets (the styles had fallen off). This was a middle flower of a side inflorescence (as indeed all the flowers in this series were in the cases in which a record had been made of the definite position), and the leaf-stalk to the auxiliary leaf of this inflorescence also showed signs of 'forking'; the leaf itself had not been preserved, but if it had shown any peculiar feature it probably would have been. The vascular structure of this petiole is shown in Fig. 1, *h*, and may be compared with that of the normal petiole of Fig. 1, *g*.

The next series (see Table II) was collected on the chalk near High Wycombe, late in September and early in October, 1917. There is only one record of an abnormal side flower. Its formula gives an idea of its nature: $K_4, C_1 + 4, A_2, G(2)$. A similar flower has this formula in my notes— $K_3, C_2 + 3, A_2, G(2)$, but it is not recorded whether it is a side or a middle flower. All the others were middle flowers. Of these, two showed five stamens, in one case with a forked upper lip (Fig. 2, *c*), and in the other with an upper lip resembling a side lobe of the lower lip; another two showed upper lips similar to the corolla; in one of these there were four sepals and in the other six.

TABLE II. SERIES B. *Downley. September-October, 1917.*

	K.	C.	A.	G.	N.	Remarks.	Fig. 2.
1. f.	10	1+3	7 or 8	2+3	...	M.F., 1 N.S.F.	...
...	...	1+3
2. f.	10	(1+3)	8	2+2	...	M.F., 1 N.S.F.	...
...	...	1+3
...	...	(1+3?)
3. f.	10	the rest missing	M.F., 1 N.S.F.	...
4.	5	2+3	5	2	...	M.F.	<i>c</i>
5.	5	1 s.p. + 3	5	2	...	M.F.	...
6.	6	1 s.p. + 3	4	2	...	M.F.	<i>d</i>
7.	4	1 s.p. + 3	4	2	...	M.F.	<i>b</i>
8.	4	1+4	2	2	...	S.F.	...
9.	3	2+3	2	2

1 = opposite half-verticillasters. N.S.F. = normal side flower. 1 s.p. = upper lip like a side petal. S.F. = side flower.

The other variants recorded were fusions occurring in opposite half-verticillasters between a middle flower and a side flower; one of these free side flowers was normal, the other was unopened but possibly normal, with five sepals. On the side with the opened flower, only ten sepals were shown, the rest of the flower having fallen off. On the other side the following formula expresses the state of affairs:

$$K_{10}, C_{1+3}, A_{7 \text{ or } 8}, G(2) + (3)$$

Under C the first series of figures expresses the condition of the upper lip and the second series (after the +) that of the lower lip: e.g. here there are two lower lips with three lobes to each. There was another fusion in which there seemed to be three upper lips (not adjoining) with three groups of stamens; this was slightly injured, however, and will not be further considered. The interesting forms in this series were those in which the upper lip resembled the side lobe of the lower lip (Fig. 2, *b* and *d*), for these must be regarded as transitional to the peloric and semi-peloric forms which have been found on chalk in July and two of which have been noticed, in September, 1919, on plants growing on a loam. These will be described below. There was a tendency to reduction in the forms

mentioned above and also in the forms with two stamens. The fused forms are interesting in that they were found in opposite side inflorescences, a distribution that is very common, as was shown by observations made in 1919, and one that probably points to similar external factors acting at the same time in the growth of the side inflorescence, bringing about a similar condition. (Fig. 2, *a*, shows an abnormality similar to that described by Muth in *Salvia pratensis* (28).) Variants were seen both at Haslemere in 1917 and near High Wycombe in the autumn of 1918, but no records were kept of them.

TABLE III. SERIES C. *Shoreham (Kent). July, 1919.*

	K.	C.	A.	G.	N.	Remarks.	Fig. 3.
1. ^a f.	9	1 + 3	7 or 8	2†	...	M.F.	k'
2. ^a f.	8	1 + 3	M.F.	k''
3. f.	10	1 + 3	M.F.†	k'''
4.	5	2 + 3	2 + 2 + 1	...	4	M.F.	...
5.	5	1 + 2	3	2	...	S.F.	...
6.	4	1 + 2	3	2	...	S.F.	e
7.	5	1 + 1	2	2	f
8.	4*	1 + 3 s.p.	4	2	...	S.F.	...
9.*	4	5	5	2
10.*	5	1 + 3	4	3	...	M.F.	a
11.*	6	6	4	2
12.*	5	5	4	2	...	M.F.	c
13.*	5	1 + 3	5	2	...	M.F.	d
14.*	4	4	4	2	...	M.F.	b
15.	5	1 + 3	4	2	...	S.F., a.i., s.e.	g

* = symmetrical flowers. a.i. = anthers in tube. s.e. = stigma beyond corolla tube.

In the middle of July, 1919, at Shoreham (Kent) (see Table III), six peloric and semi-peloric forms were found; four of these are known to be middle flowers of the lowest stalked side inflorescence and the others are thought to have been middle flowers of side verticillasters. The symmetry was usually destroyed either numerically or, as in two cases, by the presence of an upright upper lip (Fig. 3, *a*). In one of these cases a small flower was found posterior to the semi-peloric form (Fig. 3, *a*), and in the autumn of the same year, near High Wycombe, a similar tendency to form a trichasium was indicated in the case of the two semi-peloric forms then found, and also in a form with a completely divided upper lip placed in a line with the lower lip. There were three 'fused' flowers (Fig. 3, *k'*, *k''*, and *k'''*), which should be regarded as fusions between a middle and a side flower of a half-verticillaster (as indicated either by a difference in size of flowers or by one being open and the other closed), no undoubted case of fasciation being found. Fused flowers, similar to those here described, are sometimes met with normally, e.g. in certain species of the genera *Lonicera* (2) and *Eucalyptus* (49). In one of the reductions only the side petals of the lower lip were present (Fig. 3, *e*); and in another only one lobe, like a side

lobe, was present (Fig. 3, *f*). These specimens resembled two of the semi-peloric forms mentioned above, and three of the four were recorded as side flowers; the one with no record against it was probably a side flower also. There was one flower, a middle one, of a stalked side inflorescence which was pseudo-terminal owing to the destruction of the adjoining side inflorescence and of the main axis. The flower developed in an almost symmetrical manner and had an increased number of parts (Fig. 3, *e*). The remaining variant (Fig. 3, *g*) had its upper lip in line with the lower lip. The style was in line with the corolla tube, and the stamens, four in number, did not show above the tube. Flowers like this one were comparatively common in the autumn.

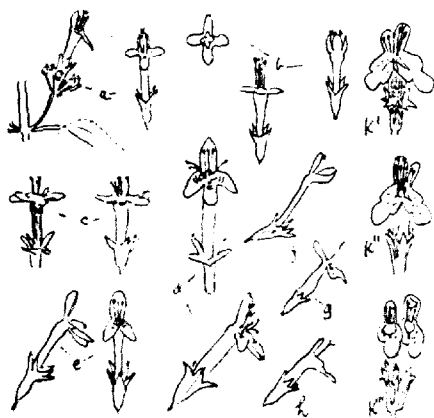


FIG. 3. For description see Table III and text. [Shoreham, Kent, 1919.]

In the middle of September of 1919 (see Table IV), on loamy soils near West Wycombe, only two symmetrical (peloric) flowers were found. a few fused flowers, some with the upper lip half fused with the right or left side petal of the lower lip, a comparatively large number of the type of flower described last in the preceding paragraph, i. e. small flowers with stamens never growing beyond the tube (Fig. 4, *d, c*), and some of these with shortened styles as well; an examination of these flowers preserved in alcohol showed either no signs of pollen grains or small, shrivelled, and probably abortive ones. An examination with a hand-lens in the field had suggested already that many of these anthers were aborted. The ovaries seemingly were not well developed, but I was unable to make out whether they were abortive as well. The evidence available seems to show that there was a tendency towards the development of gynomonocism. The

styles and stigmas were well developed except in a few forms in which the style reached *only* to the mouth of the tube.

TABLE IV. SERIES D. *Naphill* (near High Wycombe). September, 1919.

	K.	C.	A.	G.	N.	Remarks.	Fig. 4.
1. f.	8	7	7	2+2	4+4
2. f.	9	1+3	3	2
...	...	1+3	3	2	k
3. f.	9	1+3	4	2
...	...	Unopened side flower		k
4.	5	2+3	4	2	...	M.F.	g
5.	7 or 6+1	1+3	4	3 or 2+1	...	M.F.	h
6.	5	2+3	4	2
7.	5	2+3	4	2	...	M.F.	b
8.	5	1+1	2?	2
...	...	2
9.	5	1+1	3	2	...	S.F.	...
...	...	2
10.	4	1+2	3?	?	4	S.F.	...
11.	5	1+1	3	S.F.	...
...	...	2
12.	5?	1+3	2?	2
13.	4	1+1	2+1	1	...	M.F. small	...
...	...	2
14.	5	1+3	3 or 4	2	...	M.F. small	e
15.	4	4	4	2	...	M.F.	f
16.	4	4?	4?	2	...	S.F.?	...
17.	5	1+3 s.p.	4	3	...	M.F.	...
18.	5	1+3	4	a.i., s.e.	...
19.	5	1+3	4	2	...	a.i., s.e.	...
20.	5	1+3	4	2	...	a.i., s.e.	...
21.	5	1+3	3+1	2	...	1 a.e., 3 a.i., s.e.	...
22.	5	1+3	4	2	...	a.i., s.e., M.F.	d
23.	5	1+3	4	2	...	a.i., s.e., M.F.	d
24.	Opening flower undeveloped A. and G.	...
25.	5	1+3	3+1	2	...	1 a.e., 3 a.i., s.e.	...
26.	5	1+3	4	2	...	a.i., s.e. anthers M.F. (shade) (sterile).	...
27.	5	1+3 s.p.	4 (hairy)	2	...	M.F. a.i., s.e. small	...
28.	2	...	a.i., s.e. similar	...
29.	M.F. a.i., s.e.	...
30.	M.F. a.i., s.e.	...

A number of forms (Fig. 4. a) were also found with short, wide corolla tubes, short filaments, abortive pollen, as far as could be found out from the alcohol material, and styles which were commonly held in the lower lip and had the stigmas dipping into pollen which had possibly dropped from the anthers into the depression in the lower lip. No evidence was found to show whether this self-pollination was effective or not. If all the pollen of the flowers was abortive it could of course have no effect, but if some of it was capable of germination and of pollinating other flowers, there could be no *a priori* reason against its being effective on the pistil of the flower to which it belonged, for Knuth (23) reports self-pollination as usual in the ordinary flowers of this species.

Similar flowers to these (and certainly not fertile) were formed in the summer in large quantities in plants that were obviously diseased, as they

were attacked by *Perrisia Stachydis*. Very few galls, however, were found in the autumn (Connold does not report the occurrence of this gall so far on in the year), and the flowers did not show obvious signs of disease, as did those found in the summer. It is possible that such self-pollinating flowers are due to malnutrition.

Other flowers were also found in which pollination had taken place in the unopened bud. There were comparatively few of these; and it was thought, at first, that the anthers had been accidentally made to dehisce when splitting open the flower. A careful examination of artificially opened flower-buds, however, showed that this was not the case. Bud-pollinated flowers differed from the ordinary buds only in having open anthers and widely divergent stigmas, with pollen on them; such flowers could not be distinguished from the ordinary flower-buds except by opening them.

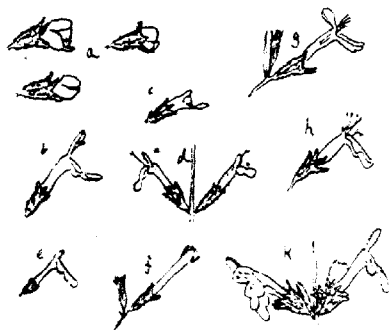


FIG. 4. See Table IV and text. [Bradenham, near High Wycombe, Bucks., 1919.]

Goebel (17) has figured flowers of *Lamium amplexicaule* which are similar to the ones here mentioned. I have no evidence to bring forward as to the conditions under which such forms develop. They seemed to be fewer after rain; but few such flowers were found, and I have no information as to the relative frequencies before and after rain.

Near this district, on the chalk, three groups of plants bearing virescent flowers were found (Fig. 5), two groups in a hedge and one just on its borders. All the flowers on these plants were affected; but all other clumps of plants in the immediate neighbourhood, on either side of the hedge, were in every way normal. No sign of any disease, nor of anything that could be related causally to the condition, could be found either in the parts of the plant above ground or in the underground portions. Plants have been gathered and replanted in the hope that further observations might give a clue to the reason for this condition. A note by A. W. Bartlett (8) gives a very careful description of the condition of the gynoecea in two

abnormal specimens of *Stachys sylvatica* collected by him, and this description applies also to the plants mentioned above. But my specimens also showed an increase in the size of the calyx, a greening of the slightly reduced corolla, varying from slight strips of green to an almost completely green, and the anthers were affected as well. The flowers were abortive through the phyllody or sepalody of the carpels. The average length of the calyx tube in hedge forms was found to be about 4 mm.; the average length of tube and medium-sized free portion of sepal was about 6.5 mm.; the similar figures for forms growing in woods were 3.5 mm. and 6.7 mm., while the figures for the calyx of virescent forms were 4.6 mm. and 8 mm. The filaments remain short, the anther lobes more or less symmetrical (Fig. 5 *b*), and the pollen grains are wrinkled and seem destitute of contents. I have

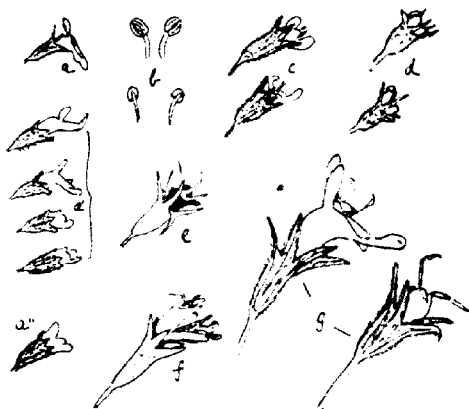


FIG. 5. Virescent flowers from Downley, near High Wycombe, Bucks., 1919-1920. [See text.] *e, f, g* slightly enlarged.

very little doubt but that they were abortive. Molliard (26) reports cases of virescence due to animal parasites, and similar cases have been reported by Butler (7) as due to fungal infection, while de Vries (43) has investigated cases in which the tendency to form virescent flowers was inherited, and was intensified under the conditions of his cultural experiment.

In September, 1920, this region was again visited; virescent plants were found in the same spot, as well as farther along the hedge. The hedge was mostly of hawthorn, but elder plants were fairly abundant. Normal plants were also found growing here. In some of the virescent shoots the four green nutlets had increased in size enormously and showed well above the enlarged calyx (Fig. 5, *d*), and in other shoots occasional flowers were found in which the ovary had been partially changed into a flower or into two flowers (Fig. 5, *e, f, g*), and in the case of other ovaries a flower

was found to replace the contents of the ovary. Cases were found in which the ovary had been changed into two leaf-like structures, but they were never so much like a leaf as those that Bartlett figures. There seems little doubt that the condition is carried over from year to year vegetatively. The plants here shortly described are probably similar to those mentioned by Townsend in his 'Flora of Hampshire' as var. *viridiflora*, and regarded by him as abnormal.

TABLE V. *Statistical Examination of Open Flowers in the Autumn of 1919 (near High Wycombe).*

Int.	Total.	Normal.	Abnormal.	V.	M.	C.	S.	R.	F.	Y.	X.
<i>Blacksmith's Lane:</i> (light period from 4 p.m.)											
17th.	144	128	16	2 (M.)	4 M	2 M	M	...	M
...	...	[51 (M.) 77 (S.)]	4 S	2 S	(s.p.)
19th.	298	287	11	7 (S.)	M	2 M	M
...	...	[106 (M.) 181 (S.)]
20th.	326	269	57	14	7 M	2 M	M	3 M	M
...	...	[124 (M.) 165 (S.)]	...	5 (M.) 9 (S.)	6 S	S	...	2 S
<i>Blacksmith's Arms Lane:</i> (light period from 11.30 a.m.)											
17th.	98	96	2	2 (M.)
...	...	[35 (M.) 61 (S.)]
<i>Plantation Hedge:</i> (light period until about 4.30)											
16th.	237	223	14	4 (S.)	3 M	2 M	M	...
...	...	[96 (M.) 127 (S.)]	2 S	S	...
<i>Leam Hedge:</i>											
18th.	233	210	23	18	3 M	M	...
...	...	[82 (M.) 128 (S.)]	...	[6 (M.) 12 (S.)]	1 S
<i>Vigilant Chapel Road:</i> (light period from about 4)											
18th.	54	54
...	...	[17 (M.) 37 (S.)]
<i>Langstone Corner:</i> (light period until about 2 p.m.)											
17th.	98	98
...	...	[58 (M.) 60 (S.)]
<i>100' Hall Wood:</i>											
17th.	20	19	1	S
...	...	[1 (M.) 18 (S.)]
<i>Field, Ash High Road:</i> (light period until about 11.30)											
17th.	44	39	5	5	2 M	3 S
...	...	[10 (M.) 29 (S.)]
Totals	1,532	1,443	109	52	32	7	1	5	3	5	4

M. = middle flower. S. = side flower. V. = bifurcated upper lips. C. = only slightly opened flowers. Reduced number of parts. s. = symmetrical flower. X. = unclassified abnormal. Y. = lower lip of corolla divided.

It should be mentioned here that a variety of *Stachys sylvatica* has been found at Shoreham which differs from the type mostly in that there are no markings on the lower lip of the corolla. This plant will be described elsewhere. There is no evidence that any of the plants described here are hybrids.

After observing bud-pollination and the forms with abortive and reduced stamens, it was determined to obtain information regarding the frequency and distribution of the respective variants. Several places were selected, in the same neighbourhood, where the *Stackys* was growing in quantities and the number of normal and abnormal open flowers were noted, in every case examining the flowering shoot from below upwards and distinguishing between the middle and side flowers of the side dichasia. The areas selected were typical ones, mostly hedgerows, and differed especially in the direction they faced, and therefore in the length of their daily exposure to sunshine. In the two sites provided with the greatest illumination (*e* and *c*) there were practically no variants but those with forked upper lips; and in another area (*b*), where the lighting was very good and the hedge plants had recently been cut (a few days before counting, no open flowers were present, and a week before no signs of inflorescences could be seen), only two variants were found. The wood records are not typical, as there were few flowers remaining in such shady situations. The percentage of middle flowers with stamens having short filaments will be found to be much smaller than the percentage of side flowers in a similar condition. This result, however, is deceptive. Flowering shoots bearing such flowers were very much reduced, not so much in the number of flowers borne as in regard to the length of the 'internodes' and in the size of the flower; most of the corollas never opened, but withered *in situ*, and the greater number of them did not develop their nutlets to maturity. In fact no information was obtained as to whether the fruits of such flowers ever matured. The majority of such side flowers, therefore, probably never open, and their condition was not considered in the above numerical statement, nor was the frequency of bud-pollination recorded.

In June–July, 1920, plants were again examined on the chalk in Kent. Fusions and symmetrical flowers were commonly found, and a few flowers with reduced parts, i.e. two lobes to lower lip of corolla or with three similar lobes to lower lip of corolla. These latter cases were always the last-opened flowers on side verticillasters which were usually in a low position on the plant, and their condition can be taken to be due to deficient nutrition. In all the very numerous cases in which the corolla tube was wide open and the stigmas were bent over and fitting into the lower lip of the corolla, there were undoubted signs of disease, and the 'grub' itself was seen low down in the corolla tube in nearly every case. The fusions were difficult to treat statistically, for in some clumps of the plant there would be no abnormals of any kind, in other regions there would be a few of various sorts including a fusion, and in other places again there were plants showing a large number of these negative *dédoublements* and, as far as could be discovered, little else that was abnormal. Counts were made in one such patch; all the plants were affected and nearly all were examined statistically.

those counted being taken at random. Comparatively few open flowers showed fusions, and in the counts all signs of fusing, whether in bud, flower, or fruit, were made note of (the half-verticillasters were counted and not individual flowers.)

Plants.	Total half-verticillasters.	Normal half-verticillasters.	Fusions.	Normal middle verticillasters.	Fusions.	N. side.	Fusions.
13	422	332	90	162	87	168	

It will be found that most of the fusions were in the middle half-verticillasters, and but few on the side compound inflorescences; nearly all the fusions were between two flowers, but three were between three flowers. The only thing noticeable that was remarkable in the position of these plants was that they had formerly been overshadowed by a row of elm-trees growing on the opposite side of the road. The trees had been removed recently (in December, 1919).

The symmetrical flowers are found much more rarely, but in all kinds of situations, and in hedgerows facing in any direction. They are in every case apical flowers of side compound inflorescences.

The smaller *Stachys* shoots bear a series of opposite pairs of half-verticillasters arranged in a racemose manner; larger shoots, in the pair of leaves just below this simple construction, will be provided with a similar pair of racemously arranged verticillasters, and in plants that are even more strongly developed there may be several such structures.

In fairly well developed plants, with the simplest construction, the flowers on the lowest verticillaster will be longer stalked than usual, and in the cases of symmetrically constructed flowers, that I have already described, it has been noted that the half-verticillasters bearing them are a little more complicated than usual and approximate to trichasia.

In the symmetrical forms examined this summer it seemed clear that a series of transitions could be found between the simpler form of dichasium through the trichasium to the cases where, in a similar position, were to be found a series of verticillasters arranged in a spike-like manner. It is in these transitional forms of inflorescence that symmetrical flowers are to be found; such flowers are older than the flowers immediately below them, and in many, if not in all, of the plants examined by me stand in the relation of first or older dichasial flower to the flower above it, i.e. next the main upright shoot—and also to the one below it, i.e. on the side away from the main upright shoot.

This apical flower often partially fuses with one of the side flowers of this dichasium, and then, although it can be seen that the plan of the flower was on a symmetrical pattern, the fused structure is contorted to one side and it is very difficult, if not impossible, to distinguish the parts, the corolla tube sometimes being open and fused with the calyx cap, which is also split. It seems likely, then, that the symmetry is brought about when the lowest

inflorescence is passing through a series from the compound spike to the simple dichasium, and at the particular point when either a trichasium is found or when there is a top flower to a fairly complex inflorescence bearing side flowers in a dorsal and ventral position.

I can offer no opinion as to the external conditions which control the formation of these transitional forms of inflorescence.

In one case an almost symmetrical side flower was found, but even here it was a part of a trichasial system.

It will be remembered that Peyritsch (31), in his various papers on the cause of peloria, was of the opinion that in several Labiatae sudden changes in illumination induced this condition, and that he brought forward a certain amount of experimental evidence pointing in this direction. I have found peloric flowers in *Galeobdolon luteum*, the plant experimented on by Peyritsch, in a hedgerow which had been greatly depleted of its shade.

Last year (1920) plants collected in the neighbourhood of Radlett, Herts., were planted in my gardens, some in the shade (under a tree) and others in a more open situation. As would have been expected, the shade plants flowered (i.e. bore open flowers) later than the sun ones, indeed about a week after—those in the sun flowering on May 24. and those in the shade on June 1. The general condition of the shade flowers was unsatisfactory; they often withered before they opened; they were stunted, and, so far, one case of fusion has been observed amongst them. On a shoot, on which but few flowers were open and those towards the light, a flower was found with a bifurcated upper lip.

The sun plants seemed, on the whole, normal, but one plant bore two flowers with lower lips having the middle lobe like the side ones (with two lobes instead of three to the lower lip). One of these flowers was a late developed one in the lowest verticillaster, and the other was found on the third verticillaster in a similar position.

DISCUSSION.

There are a large number of observations showing that external conditions (time of year, good or bad nourishment) and the position, advantageous or otherwise, of the inflorescence on the plant have an effect on the number of flowers in a capitulum in the case of the Compositae (35) and on the number of members in the whorls of individual flowers in many other cases. MacLeod (25) found, for example, that in some plants of *Ranunculus ficaria* the percentage of members in a whorl was smaller under adverse circumstances. Correns (8, 9) obtained similar results in *Satureia hortensis*; Burkill (6) reports that in *Bocconia* and many other plants examined statistically by him the flowers in favoured positions on the inflorescence had a larger number of sporophylls, and Salisbury (33) recently notes that this is the usual, though not the invariable, state of affairs in the case of the

carpels of *Clematis Vitalba* Goebel (18) mentions that in the Caryophyllaceae the first flowers to open are often hexamerous, while the later ones are pentamerous, and that in *Ruta graveolens* the terminal flowers of the cyme are pentamerous, the others tetramerous. A survey of similar cases can be found in Vernon's 'Variations in Animals and Plants' (40) and in de Vries's 'Mutation Theory' (43). The position of the flower together with a reduced number of parts in *Stachys sylvatica* suggest that bad nutrition is the causal factor, and it is probable that some cases of increased numbers of parts are due to increased nutrition, though some of these cases are probably the results of fusions between two or more flowers, and an explanation must be sought for these by considering the causes of fusions in *Stachys* flowers.

The cases where five stamens develop, instead of the usual four, cannot be regarded as infringements on the 'law of loss' recently put forward by Dr. Agnes Arber (1), for in the allied *Stachys recta* Payer (29) described five rudiments of stamens, four of which develop. In the cases mentioned above it is possible that the posterior rudiment develops instead of aborting.

The decrease in number of members in the corolla seems to take place independently of the time of year. An increased number of petals seems commoner in summer, and the change in the number of members in the androecial whorl, if not accompanied by any change in any other part of the flower, seems to be more dependent on the time of year than on the position of the flower in the inflorescence.

As regards the fused flowers, it is difficult to assign any particular reason for the phenomenon. Worsdell (49) truly says that it is not surprising that such an occurrence is common amongst the Labiatae because the flowers are so very near to each other. The actual number of flowers is not of much importance, for in such species as *Ballota nigra*, where the number of flowers in a dichasium is very much greater than in *Stachys sylvatica*, fusions are rare—partly, no doubt, owing to the longer flower peduncles—and in *Stachys* itself the fusions do not seem to be found in greater numbers in portions of the plant where most flowers are found in a verticillaster; and not only so, but where a fusion is found there is sometimes no fusion in the opposite half-verticillaster, even if the latter contains more flowers than the former. The fusion, indeed, seems to be mostly conditioned by the non-development of the flower peduncle. Some check to its formation must happen just before the floral organs are about to be laid down. It has been noticed that in depauperate forms not only are the numbers in a whorl smaller than in the normal species, but that they are often fused. In very small scapes of *Ophrys apifera* I have found small flowers without the two posterior petals and with the posterior sepal fused with one of the side sepals, and similar cases have often been described elsewhere. Also in *Fraxinus excelsior* vegetative buds, that would otherwise only develop next spring, if at all, often open, in late summer or the

beginning of autumn, as a consequence of injury to the apical bud; in such cases, structures that would ordinarily be developed as scale leaves are formed into transitional leaves. Such structures, if situated in the anterior-posterior plane, have enlarged bases which are quite free; if, however, they are placed right and left of the shoot, the bases are generally fused on the side towards the parent shoot, but free on the other side. It is possible that some comparable nutritional disturbance is responsible both for the fusion between flowers in *Stachys*, and for the occasional bifurcation of the upper lip of the corolla.

The tendencies to *bud-pollination* and *gynomonoecism* were shown only in the autumn. The work of Vöchting (41, 42) has indicated that in *Mimulus* light is the controlling factor in cleistogamy, and in other plants temperature also has been shown to be important; while Graebner, in 1893 (19), has expressed the opinion that cleistogamy is brought about by any adverse influence, e.g. lowering of temperature, amount of light, or the weakening action of fungi. Willis (45-48) is in agreement with this view, and also expresses the opinion that the peculiar distributions of the sexes seen in the Labiatae and other orders are also expressions of the action of external adverse conditions. It is not known precisely what set of external conditions favour cleistogamy rather than gynodioecism or androdioecism. It is possible that the time of action of the factor relative to the stage of development of the plant or flower must be taken into consideration. The observations of Delassus (11, 12) and of Urbain (39) are here of some importance, for they have shown that the effects of partial or complete removal of the endosperm or of fleshy cotyledons are felt far into the life of the plant, influencing the time of flowering, the number of flowers, and in some plants producing frequent sexual abnormalities. This fact makes the investigation of any such phenomena difficult, for it is not an easy matter to trace back any anomaly to its cause, and the case of *Stachys* would be especially difficult as, when in bloom, it has many flowers in different stages, and these flowers react on one another, the development of one flower often causing the non-development of another by correlation.

Klebs's work on the importance of light in bringing about flower formation (22),¹ and especially the extension of this view by which it is held that an excess of starch-formation is responsible for flowering and an excess of salts for vegetative development, impresses on us the presence of quantitative factors as apart from qualitative ones. Sprecher's results on *Rumex* and *Cannabis* (36), in which genera he finds that in the different sexes there are different osmotic pressures in the expressed juices and that the mineral and organic matters present in the extract are also not the same, point to conclusions similar to those to be drawn from Klebs's work. It is true that several workers have held that the sex in hemp and similar plants is fixed

¹ See also Garner and Allard (14), and Giron de Bazeingues (16).

from the seed and that nutrition has no effect in altering it. Haberlandt (20), Heyer (21), and Fisch (13), have expressed this opinion for this particular plant. Other workers, however, have shown that mutilation and changed conditions can bring about a change of sex in hemp (Pritchard (32), Schaffner (34), Tournois (38)). Similar observations have recently been recorded for *Myrica Gale* (Davey and Gibson (10)) and for *Plantago lanceolata* (Stout (87), Bartlett (4, 5), Ludwig (24)); while Yampolsky has not only given us the results of his own work on *Mercurialis annua*, but has provided us with a summary and review of previous work on so-called intersexes (50, 51, 52).¹

The fact that *Stachys* plants, in the autumn, bear a certain number of female flowers makes an addition to the number of plants in which it has been shown that the development of 'sporophytic' sex is dependent on external conditions.

SUMMARY.

1. During the months of June, July, August, September, and October, plants of *Stachys sylvatica* have been observed, showing peloria, semi-peloria, fasciations, synanthly, chloranthly, increase and reduction in number of parts of all four whorls, a tendency to abortion of the stamens (gynomonoecism), and 'bud-pollination'.

2. The semi-peloric flowers were apical flowers on the more important side inflorescences, and their position on the plant was rendered more symmetrical, either by injury to the main stem or by the development of a trichasium through the formation of flowers between the peloric flower and the main axis.

3. An increase in the number of members in a whorl must probably be connected with increased nutrition, and a reduced number with conditions inimical to good nutrition.

4. The abortion of stamens is accompanied by a marked decrease in size of calyx and corolla, the smaller side flowers withering without opening. Such flowers were found mostly in the autumn.

5. The variants noticed in the autumn were fewer in warm and sunny situations and were mostly of the nature of a bifurcation in the upper lip of the corolla. In two regions practically no departures from the normal were noticed; and in the case of one of these regions, which was well lit, the flowers were all borne on new shoots and were, therefore, probably making use of the food stores of the underground rhizomes.

6. Early in the year the tendencies exhibited are towards an increase in the number of parts, especially in the androecium and gynoeceum, and an exhibition of fasciations. Such features are usually shown in the middle flowers. During the whole of the year notched upper corolla lips were

¹ See also Giard (15) and Wester (44).

common, but no statement can be made regarding their relative frequency. Fusions between a middle and a side flower were more commonly found and are difficult to distinguish from fasciations.

Flowers with a reduced number of parts in the corolla are common all the year, and these are found to be side flowers. In September a tendency to gynodioecism and possibly to self-pollination is shown, and 'bud-pollinations' occur. It is not known whether self-pollination, under these circumstances, is effective or not.

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A Fourth Contribution to our Knowledge of the Anatomy of the Cone and Fertile Stem of Equisetum.

BY

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With Plate XXI and twelve Figures in the Text.

I. MATERIAL.

IN the course of the present investigations the cones of three species, *E. sylvaticum*, L., *E. debile*, Roxb., and *E. variegatum*, Schleich, were studied. Of *E. sylvaticum* three cones, A, B, and C, were cut serially into transverse sections. In the cases of Cones A and B, the sections extended to a level just below the annulus, but in Cone C the series ended just below the insertion of the lowest whorl of sporangiophores. A fourth cone, D, was cut serially into longitudinal sections.

Five cones of *E. debile*, henceforward referred to as Cones A, B, C, D, and E, were cut serially into transverse sections, and a sixth cone, F, was cut into serial longitudinal sections. Below Cones A, B, and C the series of sections was continued so as to include the uppermost node of the cone-bearing branch, while below Cone E the series of sections extended to a point just below the second node in a downward direction.

Of *E. variegatum* serial transverse sections were obtained of three cones, A, B, and C. Below Cone A the series of sections was prolonged, so as to include two vegetative nodes. Of a fourth cone, D, serial transverse sections were made of the region transitional from annulus to cone.

The following table gives particulars as to the size and constitution of the cones. In all cases, except in that of Cone C of *E. sylvaticum*, the

Species.	No. of whorls.	No. of sporangiophores.	Height of stipe.	Greatest width of stipe.
<i>E. sylvaticum</i> . A.	13	162	circa 18.8 mm.	circa 1.5 mm.
" " B.	11	116	" 18.7 "	" 1.3 "
" " C.	14	149	" 17 "	" 1.28 "
<i>E. debile</i> . A.	9	68	" 10.5 "	" 1.5 "
" " B.	9	60	" 5 "	" 0.46 "
" " C.	6	38	" 8.2 "	" 0.6 "
" " D.	6	32	" 7.25 "	" 1.4 "
" " E.	7	38	" 9 "	" 0.75 "
<i>E. variegatum</i> . A.	5	35	" 2.5 "	" 0.6 "
" " B.	4	25	" 2.4 "	" 0.5 "
" " C.	4	25	" 2.4 "	" 0.45 "

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height of the stele was measured from a point just below the insertion of the annulus to the level, above the last whorl of sporangiophores, at which the vascular structure characteristic of the extreme apex of the cone is established.

II. GENERAL DESCRIPTION.

The cone of *E. sylvaticum* more closely resembles in its anatomy the cones of *E. arvense* and *E. maximum* than those of the other species studied. The resemblance is closest to the cone of *E. maximum*. The radial extent of the axial xylem is slight; except in the neighbourhood of the rather more deeply seated groups of protoxylem, the metaxylem is usually only one to two (often only one) cells in depth. Moreover, a few parenchymatous cells are not uncommonly interspersed in the tracheides of the metaxylem, though in the species under consideration this intercalation of parenchyma among the tracheides is not as extensive as in *E. maximum*. Nevertheless, in a transverse section of the cone of *E. sylvaticum*, the general resemblance to the cone of *E. maximum* is striking, especially, of course, to the smaller specimens of the latter species. The wide stele, the relatively numerous strands, the small radial extent of the metaxylem, its interruption by parenchyma, the small size of the tracheids, the presence and distribution of the tannin cells, all these characters in combination vividly recall the cone of *E. maximum*. But, relatively to its size, the cone of *E. sylvaticum* has a better developed vascular system. For instance, in the cone of the latter species the protoxylem generally abuts throughout most of its course on the metaxylem, whereas in *E. maximum* a few parenchymatous cells intervene in the internode of the cone between protoxylem and metaxylem (cf. Barratt, p. 224, Text-fig. 21).

Though typical endodermal markings are not well shown in the axis of the cone a common outer endodermis can be distinguished, comparable to that of the ordinary stems. There was no trace of a common inner endodermis, such as Pfitzer found in the rhizome (Pfitzer, pp. 313-14).

The tracheides of the cone of *E. debile* are of the usual type. Though bands of xylem occur locally (cf. Text-figs. 3-6) many of the strands are very narrow (Pl. XXI, Fig. 7). In mature specimens, such as Cone A, it is often the case that some of the bundles in a transverse section of the axis have well-marked carinal canals, perhaps nearly half the size of the bundle, while in their immediate neighbours the disintegration of the protoxylem is only beginning. The metaxylem varies a good deal in amount. It is usually narrow laterally, so that the strands are widely separated and parenchymatous cells are interspersed among the tracheides. There is sometimes a tendency for tracheides obviously belonging to the metaxylem to be almost or quite as deeply seated as the protoxylem (cf. Pl. XXI, Fig. 7, especially the large bundle). When the strand is narrow its outline tends to

be more or less circular or even oval with the slightly longer axis directed radially. But, though the radial extent of the xylem is often considerable, compared with its width, the actual number of tracheides in the metaxylem of the small bundle is not great and a comparison with the bundles in younger, immature cones shows that the disorganized protoxylem elements, replaced by relatively large carinal canals, were few in number. Protoxylem and metaxylem are usually continuous, but may be locally separated by parenchymatous cells.

The individual strands of the axis of the cone are closely surrounded by a sheath, often very distinct, the cells of which do not show typical endodermal markings. They are much larger than the cells of the bundle and are often radially elongated with reference to the latter (cf. Pl. XXI, Fig. 7). The sheath always follows closely the shape of the bundle, and when there is a band of xylem, formed by the coalescence of several strands, the cells of the sheath disappear at the points of junction of the strands, their place being taken by tracheides mixed with small parenchymatous cells. Thus the laterally elongated bands of vascular tissue are surrounded by sheaths exactly resembling those round the narrower bundles.

In the cone of *E. variegatum* the xylem also consists of tracheides of the usual type. The protoxylem is usually in contact with the metaxylem, and though parenchymatous cells are mingled with the tracheides they are not, as a rule, as numerous, even relatively to the size of the bundle, as in *E. sylvaticum* or *E. maximum*. The tracheides of metaxylem and protoxylem are often very similar; the latter may be irregularly scattered along the inner edge of the former and not distributed in definite groups. The amount and distribution of the metaxylem varies much, even in neighbouring bundles of a section. Perhaps the commonest form of strand is that in which the xylem forms an oval band, as seen in a transverse section of the axis, wider than deep, broken up by a few parenchymatous cells, but often attaining locally a depth of three to four cells, excluding the protoxylem (cf. Pl. XXI, Fig. 5).

Sometimes certain relatively large tracheides at the edge of the bundle appear to be more deeply seated than the protoxylem (Pl. XXI, Fig. 5, the bundle near the top of the figure on the reader's left). Such xylem is, ontogenetically speaking, centripetal, since its differentiation as tracheides occurred after that of the smaller celled protoxylem outside it. These relatively large tracheides abut on the large cells of the sheaths that surround the separate bundles or bands of vascular tissue and usually line one of the sides of this sheath. It is obvious from the early appearance round the bundle of a definite sheath that the cells of the latter soon attain too great a size for them to develop as ordinary metaxylem tracheides. It is, therefore, possible that when the conditions in the developing bundle are favourable to a considerable differentiation of metaxylem, the extension of

the latter outwards or laterally being hindered by the large size already attained by the cells of the sheath, the direction of lignification of any further tracheides might be bound to follow more or less the outline of the sheath inwards.¹ The disposition of these more deeply seated, large tracheides and their appearance, as we pass upwards, in continuity with the ordinary centrifugal metaxylem strongly suggest such an explanation.² But though this is the general impression left by their appearance and position, it is of course possible that these elements are the remains of ancestral centripetal xylem.

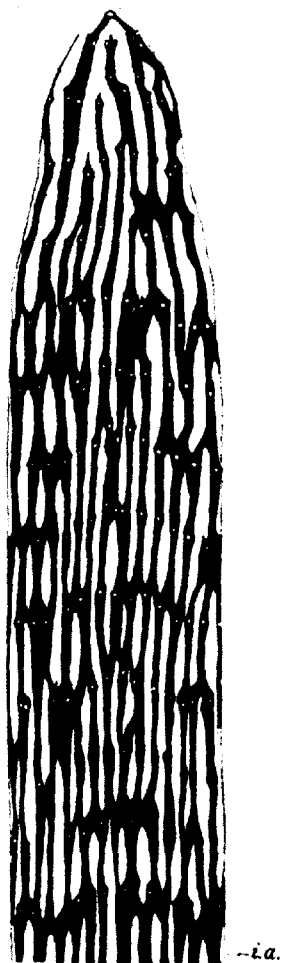
Pfitzer (pp. 310–11, 313) states that there are common inner and outer endodermes both in the stem and the rhizome of *E. variegatum*. In the cone, however, the separate bundles are surrounded by special, very distinct endodermes. When two bundles fuse, or when a strand increases in width, when it narrows or branches, the endodermis takes the outline of the vascular tissue (cf. Pl. XXI, Figs. 4, 5, and 8). Consequently the distribution of the endodermal cells is always varying, and they cannot be held to possess any morphological or phylogenetic importance.

III. THE COURSE OF THE STRANDS IN THE CONE.

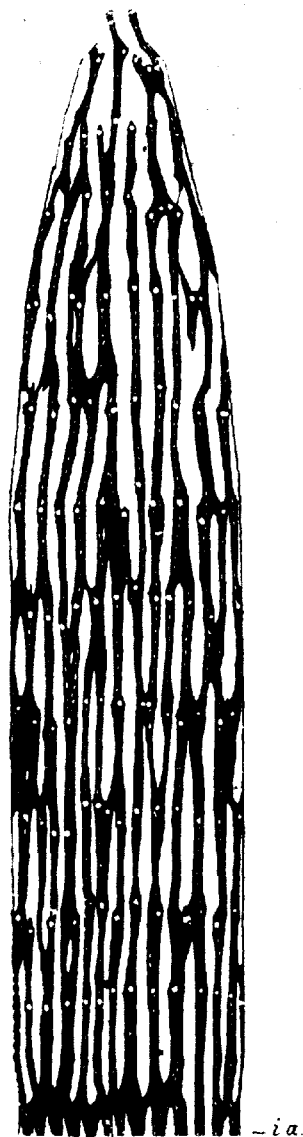
The network of strands in the cone of *E. sylvaticum* recalls that of the cones of *E. maximum*, especially of the smaller specimens with their relatively better developed vascular system. The reconstructions of the steles of Cones A and B of *E. sylvaticum* (Text-figs. 1 and 2) are very similar to the reconstruction of the stele of Cone A of *E. maximum* (cf. Browne (2), Pl. XII), allowance being made for the different scales of the figures and for the larger size and more numerous component parts of the cone in *E. maximum*. In Cone A of *E. sylvaticum* it is rather difficult to estimate the number of parenchymatous meshes that arise within the cone (i. e. above the sporangiophores), because in it the annulus is attached very close to the points of insertion of the lowest sporangiophores. The latter are disposed a little irregularly and at slightly different levels. As a result of the absence of elongation in the region between the annulus and the lowest fertile whorl the parenchymatous meshes arising by the supra-annular branching of the axial strands originate but very little below those arising above—in most cases but little above—the points of departure of the traces of the lowest sporangiophores. Thus, on a superficial examination of Text-fig. 1, two of the meshes—those shown in the reconstruction as arising between and slightly below the first and fifteenth, and the second and third traces respectively of

¹ Such an explanation would not involve the attribution of any morphological value to the sheath, since the suggested modification in the direction of development is due to a purely physiological character, the early increase in size of the cells of the sheath.

² A somewhat analogous origin of ontogenetically centripetal from phylogenetically centrifugal xylem has been suggested by Chodat (1908) for the mesarch strands of *Lysiodendron*.



TEXT-FIG. 1. Reconstruction of the stele of Cone A of *E. sylvaticum*. Axial xylem black; traces and parenchyma white; *i.a.*, level of insertion of annulus. x 8.



TEXT-FIG. 2. Reconstruction of the stele of Cone B of *E. sylvaticum*. Axial xylem black; traces and parenchyma white; protoxylem a broken line enclosing a dotted surface; *i.a.*, level of insertion of annulus. x 10.

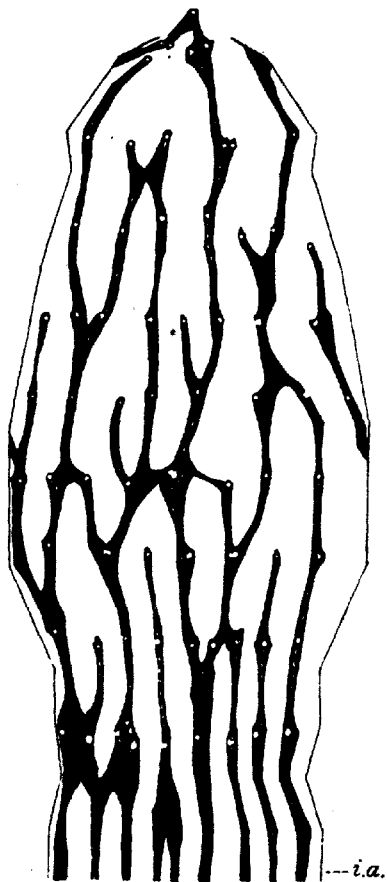
the basal whorl of sporangiophores—might well seem to belong to the first internode of the cone proper. Judging, however, from their superposition to the axial strands below the annulus, from their alternation with and from their origin slightly below the sporangiophores, these two meshes would seem to be of the same nature as those between the ninth and tenth and the thirteenth and fourteenth traces of the same whorl, the supra-annular origin of which is more obvious. The elongation of the axis between the annulus and the basal whorl of Cone B allows of a clear distinction between the supra-annular meshes and those arising within the cone proper. In Cone C this region was not sectioned.

As in all the other cones studied by me some parenchymatous meshes that originate below the sporangiophores persist into the cone. These will be considered later on. Excluding them, the following table summarizes the number and nature of the meshes found in Cones A, B, and C of *E. sylvaticum*:

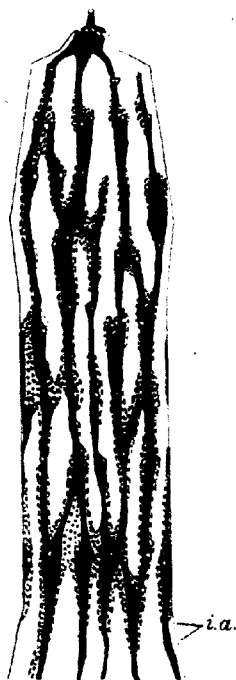
Cone.	1st order.	2nd order.	3rd order.	4th order.	5th order.	6th order.	7th order.	8th order.	9th order.	Total meshes.
A.	17	13	9	6	4	1	0	0	1	51
B.	10	13	2	2	0	2	1	0	0	30
C.	11	7	6	6	5	5	0	0	0	40
	38	33	17	14	9	8	1	0	1	121

As pointed out in a previous paper (Browne (2), p. 233), meshes of the same order may arise or be closed at very different levels in the internode. Thus, apart from the factor of the different lengths of internodes, meshes of the same order may vary appreciably in height, even in the same internode. In *E. sylvaticum* some of the meshes are closed by the formation of additional xylem in the neighbourhood of the node. In other cases the closure is effected by the oblique course of one or both branches of a strand, this oblique course leading a little higher up to fusion with another strand or branch of a strand. In such a case the mesh is only closed somewhat above the level of the departing trace and of the initiation of the new mesh arising by the forking of the strand. But, as in my previous papers, I have not considered the mesh thus closed as of a higher order than those closed at or below the node. Though the axes of Cones A, C, and D of *E. debile* have not all elongated fully, the appearance of the tissues made it clear that the differentiation of the metaxylem had been completed. On the other hand, neither Cone B nor Cone E of this species was mature. The development of the spores had not been completed, and in the cells of the sporangial wall the characteristic thickenings had not yet made their appearance. In both these cones the phloem projected beyond the xylem over a considerable vertical extent, in Cone E throughout most of the cone. To judge from mature cones, in which the metaxylem always extends laterally as far as and sometimes a little farther than the phloem, the full

differentiation of the metaxylem in these cones would have led to a further closure of parenchymatous meshes. In the reconstructions of the steles of Cones B and E of this species the phloem, where it projects be-



TEXT-FIG. 3. Reconstruction of the stele of Cone A of *E. debile*. Axial xylem black; traces and parenchyma white; protoxylem a broken line enclosing a dotted surface; i.a., level of insertion of annulus. $\times 133$.



TEXT-FIG. 4. Reconstruction of immature stele of Cone B of *E. debile*. Axial xylem black; traces and parenchyma white. The broken line enclosing a dotted surface represents the outline of the phloem, where the latter projects beyond the xylem; i.a., level of insertion of the annulus. $\times 20$.

yond the xylem, is shown by a broken outline, enclosing a dotted surface. The actual phases represented in the diagrams in no way correspond to a natural pause in the differentiation of the xylem. The transition from protoxylem to metaxylem is here very gradual.

As may be seen in Text-figs. 3-7 the vascular system of the cone of *E. debile* consists of a loose network of strands. The tracts of parenchyma are relatively wide, long, and irregular, and many of them originate below the cone proper. It may be freely admitted that an analysis of the stele of the cone of *E. debile* in terms of parenchymatous meshes, arising in the phylogeny above traces that have departed, is only possible by means of a comparative study of the anatomy of the cone in the genus. But in the light of such a study the stele of the cone appears to be constructed on the same general plan as that of the other species. This point will be further discussed later on (cf. pp. 450-1).

Leaving out of consideration the parenchymatous meshes arising below the lowest whorl of sporangiophores, we find that Cones A, C, and D, in which the vascular system is fully differentiated, consist of 68, 35, and 32 sporangiophores, and contain respectively 12, 4, and 5 parenchymatous meshes originating within the limits of the cones. In Cones A and C none of these was of the first order; but in Cone D there were three meshes of the first order. Most of the meshes become confluent with others. Usually several meshes originating separately become confluent, owing to the dying out of vascular strands after the departure of traces. This dying out of strands is not confined to cases where the following whorl consists of fewer sporangiophores. Within the limits of Cone B (Text-fig. 4) seven fresh meshes are initiated. But the development of two more in the mature cone is foreshadowed by the linking up through the phloem of (a) the strands from which the third and fourth traces of the lowest whorl depart and of (b) the strands giving off traces to the second and third sporangiophores of the third whorl. Had the differentiation of metaxylem followed, as it most probably would have in due course, the outline of the phloem three meshes (two arising below the cone and one above the second trace of the lowest whorl) would each have been converted into two meshes of a lower order. The new meshes would have been situated above the fourth trace of the lowest whorl, above the fifth trace of the second whorl, and above the second trace of the third whorl. A considerable width of xylem would also have been differentiated near the level of insertion of the basal whorl of this cone. Similarly, in Cone E the distribution of the phloem indicates that had the cone reached maturity a further development of metaxylem would probably have led, by the closure of a mesh originating below the cone, to the formation of a fresh mesh above the third trace of the lowest whorl. As it is, only one mesh actually originates within the limits of Cone E, for it seems clear that the apparent initiation in Text-fig. 7 of a fresh mesh between the second and third traces of the fourth whorl is deceptive. It is due to the transference to paper of a transient phase; for the development of the later formed metaxylem would presumably have led to the filling in of the small gap between the two

traces. The same remark applies to the small gap in the xylem between the fifth and sixth traces of the fourth whorl of Cone B (cf. Text-fig. 4).

It is characteristic of *E. debile* that a number of wide, long, parenchymatous meshes either remain enclosed throughout the whole cone (in which case the persistent meshes fuse around the base of the vascular strand of the acumen) or are only closed at or just below the apex of the cone, at the level of insertion of the closely approximated, often partially concre-



TEXT-FIG. 5. Reconstruction of the stele of Cone C of *E. debile*. Axial xylem black; traces and parenchyma white; i.a., level of insertion of annulus. x 10.



TEXT-FIG. 6. Reconstruction of the stele of Cone D of *E. debile*. Axial xylem black; traces and parenchyma white; i.a., insertion of annulus. x 10.

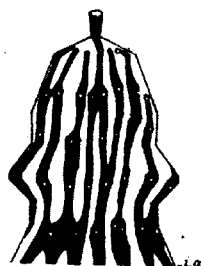


TEXT-FIG. 7. Reconstruction of immature stele of Cone E of *E. debile*. Axial xylem black; traces and parenchyma white. The broken line enclosing a dotted surface represents the outer limit of the phloem where the latter projects beyond the xylem; i.a. level of the insertion of the annulus. x 26?

cent sporangiophores of the uppermost whorl. Indeed, except in Cone D, some of the meshes persisting into the apical region originate below the cone and annulus (cf. p. 445).

Only six fresh parenchymatous meshes originate within Cone A of *E. variegatum*. All of them remain unclosed, becoming confluent with one another and with two unclosed meshes arising below the cone and persisting through it. In Cone B of this species six meshes arose within the limits of the cone and only one, of the second order, was closed. In

Cone C there were seven distinct meshes arising within the cone, and there was also an indication of what might be regarded as a minute and deferred mesh, but is a mere local failure of a few elements to become tracheides. At this point, between the second and third whorls, the xylem is only separated by a single endodermal cell, common to both endoderms. Five of the meshes are closed, two being of the first and three of the second order. At the level of the third whorl one of the strands of Cone A passes through the node without giving off a trace. A simi-



TEXT-FIG. 8. Reconstruction of the stele of Cone A of *E. variegatum*. Axial xylem black; traces and parenchyma white; *i.a.*, level of insertion of annulus. $\times 13\frac{1}{2}$.



TEXT-FIG. 9. Reconstruction of the stele of Cone B of *E. variegatum*. Axial xylem black; traces and parenchyma white; *i.a.*, level of insertion of annulus; *c.a.*, level at which axial bundles cease to present the appearance of internodal bundles of the vegetative axis. $\times 13\frac{1}{2}$.



TEXT-FIG. 10. Reconstruction of the stele of Cone C of *E. variegatum*. Axial xylem black; traces and parenchyma white; *i.a.*, level of insertion of annulus; *c.a.*, level at which axial bundles cease to present the appearance of internodal bundles of the vegetative axis. $\times 13\frac{1}{2}$.

lar irregularity has been observed in *E. limosum* (Browne (1), pp. 678-9) and in *E. maximum* (Browne (2), Pl. XIII).

In *E. variegatum*, as in the other species studied, certain parenchymatous meshes originating below the cone persist into the latter. These will be considered on a later page.

IV. THE APEX OF THE CONES.

In Cones A and C of *E. sylvaticum* the apex of the cone is traversed by a single strand, resulting from the fusion of two persistent vascular strands. At their junction these two strands enclose a few parenchymatous cells, but during the narrowing of the terminal strand, which follows rapidly on its constitution, these disappear, and the xylem forms a solid strand, more or less circular in transverse section. The structure in these cases is very similar to that found in the apices of the cones of *E. maximum* (Browne (2), p. 242). In Cone B, on the other hand, two strands lying a certain distance apart in the parenchymatous ground tissue persist into the 'drip-point' of the cone. The extreme apex of the latter had been injured, and it was not

possible to ascertain whether the two strands became confluent, but as they were relatively far apart, it is likely that they remained separate, at least for some distance (cf. apex of cone in *E. arvense*, Browne (1), p. 683).

In Cones A, C, and D of *E. debile* the stele near the apex is relatively wide, and the traces of the uppermost sporangiophores depart from two more or less widely separated vascular strands. In Cone A the two strands, much narrowed above the departure of the traces, soon fuse to form the vascular supply of the terminal acumen. In Cones C and D the narrower of the two axial strands dies out, and the other, after narrowing rapidly above the departure of the traces, enters the acumen. In Cone B the traces of the four more or less concrescent sporangiophores found at the apex of the cone depart from a closed or rapidly closing ring of xylem, recalling that found in a similar position in *E. palustre* (Browne (1), p. 683). In Cone E the distribution of the phloem indicates that had the differentiation of the metaxylem been complete a similar condition would have prevailed at the apex of the cone.

In Cones A and B of *E. variegatum* all the vascular strands except one pass out in their entirety as traces of the sporangiophores of the uppermost whorl. One strand, though giving off a trace of the same size as the others, persists and passes into the acumen. After giving off a trace it is at first very narrow, but it rapidly widens again and remains for some time markedly wider than were the strands a little lower down. In Cone C the structure of the apex is somewhat different. The tracheides of the vascular strands of the acumen, though they approach very close to one of the vascular strands of the axis, do not actually come into contact with any of them. All the bundles of the axis pass out as traces, except one, which, though giving off a trace similar to the other traces, persists as a very narrow strand. This narrow strand approaches the main vascular supply of the acumen, but does not fuse with it, and dies out before it. The structure of the apex in this specimen appears to be slightly anomalous. It seems likely that the want of continuity between the tracheides of the acumen and those of the axial strand they approach is due to an accidental and purely local failure of a few cells to develop as tracheides.

V. THE SPORANGIOPHORES AND THEIR VASCULAR SUPPLY.

The stalks of the sporangiophores of *E. sylvaticum* are relatively long and slender, and, excluding the partially concrescent members of the uppermost whorl, concrescent sporangiophores are rare, probably because, owing to the slenderness of their stalks, they are not so liable to be disposed in close proximity to one another. In Cone B, however, the first and second and the tenth and eleventh sporangiophores of the eighth whorl are respectively basally concrescent in pairs. It is possible that the occurrence in a single whorl of two pairs of basally concrescent sporangiophores should

be brought into relation with the fact that whereas the stele has diminished in width the whorl contains one member more than the whorl below.

The traces of the sporangiophores in this species are very small.¹ When passing through the cortex they usually consist, as seen in transverse section, of five to nine tracheides. In the stalk the vascular strand remains slender until close to the peltate head, where, before it divides into radiating branches, the tracheides increase considerably in number. In mature cones the lower sporangiophores are often markedly reflexed, but the traces passing through the cortex are not deflected downwards, or only very slightly so towards its outer edge. The traces, other than those of the lowest whorls, pass obliquely outwards and upwards, though all the traces show a tendency to be slightly 'bowed' near their point of departure from the axial stele. Cases in which the trace of a sporangiophore is prematurely divided, so that two separate bundles enter a sporangiophore, single in nature, are not uncommon in this species (e. g. the traces of the first sporangiophore of the fourth and of the fifth sporangiophore of the eighth whorl of Cone A; those of the tenth sporangiophore of the second, of the eighth sporangiophore of the fourth, and of the seventh sporangiophore of the ninth whorl of Cone B). In other cases the trace, though not bifascicular at its origin, arises deeply lobed and divides in the cortex or in the proximal part of the sporangiophore (e. g. the thirteenth trace of the seventh and the first trace of the ninth whorl of Cone A) (cf. p. 454).

In the reconstruction of the stele of Cone B a small white cross may be seen at the level of the second whorl. The third sporangiophore of this whorl is hardly, if at all, wider than its neighbours; but it is traversed by two independent, approximated strands. One of these is the third trace of this whorl as shown in the reconstruction; the other strand dies out in the cortex without reaching the axial stele, and the white cross marks the level at which and the radius on which its last tracheide dies out. In Cone A one of the strands passes through the level of the third whorl without giving off a trace. The tracheides of the vascular supply of the sporangiophore opposite this strand pass inwards until they come into contact with the phloem of the axis, but die out without joining on to the tracheides of the axial bundle. The phloem of the sporangiophore is continuous with that of the axis. Similar variations from the normal have, it will be remembered, been observed in *E. maximum* (Browne (2), pp. 247-8).

In *E. debile* the sporangiophores have short, relatively wide stalks. Varying degrees of concrescence are not uncommon (e. g. the fourth and fifth sporangiophores of the eighth whorl of Cone A; the fifth and sixth sporangiophores of the third whorl of Cone C; the first and second sporangiophores of the fourth whorl of Cone D, and the fifth and sixth

¹ In the reconstructions of the stele it has been necessary, for the sake of clearness, slightly to exaggerate the size of the traces of the sporangiophores.

ones of the second whorl of Cone E). Prematurely divided traces also occur (e.g. those of the first sporangiophore of the third whorl of Cone B, and of the second sporangiophore of the lowest whorl of Cone D). The traces of the third, fourth, and fifth sporangiophores of the third whorl and of the fifth sporangiophore of the fourth whorl of Cone A originate as deeply lobed bundles which fork almost at once.

The sporangiophores of *E. variegatum*, though small, have relatively long, slender stalks. Only two examples of concrescence of sporangiophores were observed outside the apical region; the seventh and eighth traces of the fourth whorl of Cone A, and the fifth and sixth traces of Cone B entered pairs of nearly completely concrescent sporangiophores. The third trace of the latter whorl supplied an unusually large sporangiophore, apparently single in nature, though intermediate in size between the double sporangiophore and the other sporangiophores of this whorl.

VI. THE REGION TRANSITIONAL FROM STEM TO CONE.

In *E. sylvaticum* parenchymatous cells are less freely distributed in the axial metaxylem in the region of insertion of the annulus (Pl. XXI, Fig. 1). Here the metaxylem is also usually of greater radial extent, and consists in part of rather wider tracheides. In Cone B, particularly, some of the tracheides in this region strikingly recall in size and form those of the nodal (or supranodal) wood, certain of them even possessing reticulate thickenings. No reticulately thickened tracheides were found at the corresponding level of Cones A and D, and the sections of Cone C did not include this region. The protoxylem is somewhat disorganized at this level, although definite carinal canals have disappeared or are disappearing. Higher up, canals, smaller and less definite than those of the internode below the annulus, make their appearance. In Cone A the region between the insertion of the annulus and the basal whorl of the cone has elongated very little, and the amount of axial xylem at the level of the departure of the traces of the lowest sporangiophores remains greater than is general in the cone, though the tracheides are less numerous, and more parenchymatous cells are interspersed among them than immediately below, where the supra-annular anastomoses occur. In Cones B and C, where the region between the annulus and the sporangiophores has elongated considerably, there is, when we reach the insertion of the sporangiophores, no unusually large amount of axial metaxylem.

In the reconstruction of the stele of Cone B a small white cross may be seen near the lower end of the sixth strand. At this point two tracheides protrude from the stele, as though they formed part of a trace. They are accompanied by two or three phloem-like and endodermal cells, oriented in the same way, i.e. with their long axis directed at right angles to those of the corresponding cells of the bundle. The cells of this incipient trace

never become free from those of the axial bundle, and a few sections higher up the latter has resumed its normal appearance.

Above the last whorl of leaves the cone-bearing branch of *E. debile* is at first smooth and unribbed. In the two axes bearing the mature cones A and C the grooving soon reappears, the ribs being in fact particularly prominent. But, unlike the other ribbed axes of *Equisetum*, the ribs are opposite the vallecular canals, and the grooves opposite the bundles (cf. Pl. XXI, Fig. 6).¹

Though Milde records (p. 135) the occurrence of stomata on the dorsal and more rarely on the ventral side of the leaf-sheaths he does not mention their presence on the annulus. In Cone B of *E. debile*, however, the upper surface of the annulus bore several stomata (Pl. XXI, Fig. 9). Though this species belongs to Milde's *Equiseta cryptopora*, characterized by a very regular arrangement of the stomata, those of the annulus appear to be few and irregularly distributed.

In Cones A, B, C, D, and E of *E. debile* the region transitional from branch to cone shows a rather wide range of variation. In Cones A and D there are, in the internode below the annulus, respectively nine and five vascular strands. These have arisen in the manner usual in the genus, i. e. by the breaking up of the ring of nodal or supranodal xylem into bundles equal in number to and alternating with the uppermost leaf-traces. But in Cone B there were only five strands throughout the greater part of the internode below the annulus, although the last whorl of leaves and the first whorl of sporangiophores both consisted of eight members. A sixth strand was, indeed, constituted, but it died out almost at once. Over two of the leaf-traces no fresh parenchymatous mesh arose, but owing to the rapid widening of the five existing meshes as they pass upwards, all the strands soon become narrow bundles, much of the same size. In Cone C of *E. debile*, where the last foliar whorl consisted of six, and the basal whorl of the cone of seven members, there were only five strands in the internode below the annulus. This is due to the fact that at the last node of the branch the ring of reticulate tracheides was not complete. A parenchymatous mesh, though markedly narrowed at the node, persisted through the latter. Consequently the traces on each side of the mesh were very near the edges of the band of xylem; above their departure the mesh between them widened again, but no fresh meshes were formed. A like failure to form a complete ring of wood at the last node of the branch bearing Cone E and a similar structure above the traces next to the persistent mesh account for the reduction of the vascular strands to four in the infra-annular internode of Cone E, in which

¹ Duval-Jouve (p. 190 and Pl. V, Fig. 13) states that in the obscurely ribbed rhizome of *E. littorale* the carinal canals lie opposite the grooves and the vallecular canals opposite the ribs. According to his description the large size of the vallecular canals appears to cause the tissues external to them to be pushed outwards, so as to simulate a blunt rib. Milde, however, after examining numerous specimens, was unable to confirm this observation (Milde, pp. 360-1).

the uppermost whorl of leaves and the basal whorl of the cone alike consist of five members. This failure to form a closed ring of xylem at the last node of the branch is clearly due to vascular reduction, not a surprising conclusion when we remember how reduced a vascular system is the network of strands constituting the stele of the cone of this species.

In Cone E the annulus, as seen in transverse sections of the axis, showed four very small groups of 2-4 tracheides running obliquely, but nearly horizontally, upwards through its parenchyma. None of these tracheides was in connexion with the tracheids of the axis, nor were the narrow, parenchymatous, phloem-like and endodermis-like cells that accompanied them and had a like orientation with them in connexion with the corresponding elements of the axial stele (Pl. XXI, Fig. 3). The latter type of cell, however, approached closer to the stele than the tracheides. As the limits of the annulus and axis are not sharply defined, it is difficult to say how far, if at all, the annular bundles penetrate the axial parenchyma. As Miss Barratt has shown that the first tracheides of the leaf-trace to be differentiated are situated distally, and that lignification proceeds in the direction of the axis (Barratt, p. 207), it might be thought that, in view of the immaturity of Cone E, these small annular bundles would later have become connected with the stele of the axis. But, apart from the fact that the specimen is not young enough to show so incomplete a stage of development, the inner termination of the annular tracheides was usually marked by the presence of endodermis-like cells, cutting the annular tracheides off from the axial stele. In the reconstruction (Text-fig. 7) these small free, annular bundles (cf. Browne (3), p. 254) are shown as small crosses, white on black and black on white, on the strands and meshes at the level and on the radius of their dying out.¹

Miss Barratt states that though the vascular structure does not support the view that the annulus is of foliar nature it cannot be said to throw much light on the morphology of that organ. The arguments she gives against the foliar nature of the annulus are, firstly, the course of the protoxylem strands in the region of its insertion, and secondly, the total absence from it of vascular supply. The latter argument she admits not to be an insuperable objection to the foliar theory, 'because the ochreola which occurs at the base of all branches always lacks traces' (Barratt, p. 228). As a matter of fact the ochreola is not always completely devoid of vascular elements,² but, since it is usually devoid of vascular supply, the absence of traces from the annulus is, as Barratt recognizes, no insuperable objection to its being regarded as a reduced leaf-sheath. Moreover, in two cones of

¹ The white cross, which should be shown on the first strand of Text-fig. 7, is unfortunately almost undistinguishable.

² Milde in 1867 recorded the occurrence of a few tracheides in the ochreola of *E. arvense* and *E. limosum* (pp. 127 and 346, Pl. II, Fig. 36), while in 1876 Janczewski indicated, in a reconstruction of a node, the presence of vascular tissue in the ochreola of *E. arvense* (Janczewski, Pl. II, Fig. 9).

E. hyemale a trace has been described passing out from the stele into the cortex at the level of the supra-annular anastomoses, though, owing to the destruction of the external tissues, it could not be ascertained whether these traces died out in the cortex or penetrated the annulus (Browne (3), p. 253). The protrusion into the cortex of two tracheides and of a few cells resembling phloem and endodermis opposite the insertion of the perfectly normal annulus of Cone B of *E. sylvaticum* may possibly be an indication of a vestigial node. The exceptional radial extent and the character of the metaxylem in this cone and the small groups of vascular cells in the annulus of Cone E of *E. debile* may also be vestigial nodal characters.

Of the course of the protoxylem Barratt writes: 'The protoxylem strands from the internode pass without any disjunction to the level at which the first sporangiophore traces arise, and hence there is no alternation at the so-called "node"'. There may be apparent forking of the strands, but this is due to the fact that the protoxylem strands in the bundles of the internode below are often double, which, separating as they enter the cone, produce this appearance of forking. This appearance is emphasized by the disposition of the metaxylem at this region, which is similar to that of the so-called nodes from which the sporangiophore traces arise' (Barratt, p. 228).

Of course, if the cases of separation of double protoxylems alluded to by Barratt occurred in specimens in which the number of sporangiophores was greater than the number of leaves at the last vegetative node little significance can be attached to them, since forking of the strands (preceded by forking of the protoxylem) is the most natural manner of effecting such an increase. Forking of this kind is found in the ordinary axes when an increase occurs in the number of members in successive whorls (Browne (2), pp. 257-8). But otherwise the presence of double protoxylem strands below the cone would seem to show that the preparation for the branching of the strand began with the branching, somewhat lower down, of the protoxylem, a very common procedure in branching bundles or meristeles. For, as Barratt points out, there is below the annulus an 'undoubted internode' (Barratt, p. 228), and we must presume that its strands arose in the usual way, above and between the uppermost whorl of leaves.¹ If so, they originated as equivalent bundles, each with a single strand of protoxylem, the position of which is marked in the internode by a carinal canal. The branchings of the protoxylem, about to be described for *E. sylvaticum*, occur slightly above the insertion of

¹ The bundles of the peduncle—as the axis between the last whorl of leaves and the annulus is called—seem typically to arise in exactly the same way as the bundles in the other vegetative internodes. I have examined a considerable number of specimens, belonging to seven species, and the only real difference that I was able to observe was that in the more reduced specimens of *E. debile* irregularities occurred, owing to the ring of xylem being incomplete at the last vegetative node (cf. pp. 440-1).

the annulus, between this level and the forking of the metaxylem strands. We may thus conclude that the forking of the protoxylem, whether it occurs somewhat below or a little above the insertion of the annulus, is probably, like the forking of the metaxylem, a reminiscence of a former node. Another explanation of the early division of the protoxylem would be that if the annulus is a reduced leaf-sheath only the free part is developed, the basal part being presumably concrescent with the axis.

In Cones A and B of *E. sylvaticum* the supra-annular anastomoses are relatively numerous.¹ Among the species studied the only one which shows a proportion of such anastomoses at all comparable to that of *E. sylvaticum* is *E. hyemale* (Browne (3), pp. 242-3 and Text-figs. 2-4). In Cone B of *E. sylvaticum* eleven out of thirteen parenchymatous meshes are closed slightly above the insertion of the annulus and eleven fresh meshes arise, one of the first, one of the second, three of the third, two of the fourth, three of the fifth, and one of the ninth orders. In Cone A of this species nine out of twelve parenchymatous meshes are closed slightly above the insertion of the annulus. As already indicated (p. 432), I interpret the two parenchymatous meshes arising slightly below and to one side of the first and second traces of the lowest whorl of this cone as supra-annular in origin. This interpretation gives five meshes as originating above the annulus, two of the first and three of the second order.

As the basal whorl of Cone A of *E. sylvaticum* consists of fifteen sporangiophores, and there are only twelve bundles in the lower part of the internode below the annulus, the forkings of the protoxylem and metaxylem in the neighbourhood of the insertion of the annulus of this specimen do not afford so good an argument in favour of the presence of a vestigial node as do the corresponding anastomoses in Cone B, where the lowest whorl of the cone consists of thirteen sporangiophores, and there are thirteen bundles in the internode below the annulus. Moreover, in Cone B the greater elongation of the region between the insertion of the annulus and the lowest sporangiophores makes the relations of the strands to one another particularly easy to follow. Though there are, of course, slight differences in size between the bundles and their carinal canals, of which each bundle possesses one, it seems clear that the thirteen bundles of the internode below the annulus are equivalent, and arose in the usual way above and between an equal number of leaf-traces given off at the whorl below. Nevertheless, but one strand of Cone B passes unbranched through the

¹ It is interesting to note that according to Milde the branches, which are produced after the maturity of the cone by the originally unbranched fertile stems of *E. sylvaticum*, often develop immediately below the annulus (Milde, p. 288). Duval-Jouve writes (p. 147) that this anomaly is rather rare in France, but is presumably much more frequent in Switzerland, since Bernoulli, always so accurate, mentions the presence of branches below the annulus as a distinctive character of *E. sylvaticum*. This phenomenon, whether rare or common, appears to be a further indication of the less great reduction of the annulus in this species compared with most others.

vestigial annular node; its protoxylem is also unbranched. Anastomoses occur between the twelve other strands, and when the bundles are reconstituted they alternate with those below the level of the anastomoses. In the cases of the two strands abutting on the two persistent meshes the alternation is of the type I have called irregular (Browne (1), pp. 681-2); but the phenomenon is very distinct. The protoxylem of the branched strands also shows indications of forking (cf. Pl. XXI, Fig. 2). In the cases of the first, fourth, fifth, sixth, seventh, tenth, and thirteenth strands of the diagram (Text-fig. 2) one of the forks of the protoxylem dies out without effecting a junction with a neighbouring fork. In the cases of the first, fourth, fifth, sixth, tenth, and thirteenth strands the very short forks of protoxylem that die out cannot be clearly shown in the reconstruction, owing to the small scale of the diagram; but in all these cases there is a perfectly distinct, short, divergent tooth of protoxylem. Both forks of the protoxylem of the third strand of the diagram and a fork of the second, seventh, tenth, twelfth, and thirteenth strands fuse with the forks of neighbouring protoxylem strands. It seems natural to regard the cases in which a fork of the protoxylem dies out without effecting a junction with a neighbouring fork as due to reduction, the reduction being greater or less according to the length attained by the fork before dying out.

In Cone A of *E. sylvaticum* three short branches of protoxylem (each one of a pair of which the other member persists) end blindly in the metaxylem above the insertion of the annulus. These short branches range from $150\ \mu$ to $400\ \mu$ in length. Since they die out in the metaxylem they are presumably unconnected with the increase in number of the sporangiophores (and consequently of the strands of protoxylem) over the leaves of the uppermost node. In these cases the forking of the protoxylem occurs a little way (about 400 – $600\ \mu$) above the insertion of the annulus, and the forks die out at about the level of and to one side of a neighbouring supra-annular parenchymatous mesh, formed by the branching of a strand.

As regards the supra-annular anastomoses the five cones, A to E, of *E. debile* show marked differences. In Cone A three of nine parenchymatous meshes are closed somewhat above the insertion of the annulus, and one fresh mesh (of the fourth order) arises. In Cone B one fresh mesh (of the fifth order) originates above the insertion of the annulus. As seen in the diagram (Text-fig. 4) none of the parenchymatous meshes of this cone was closed at this level, but from the distribution of the phloem we may conclude that had the cone reached maturity the mesh between the third and fourth strands would have been closed by the differentiation of additional metaxylem. In Cone C one of five parenchymatous meshes is closed near the level of insertion of the annulus. No clear case of the origin of a supra-annular mesh is found in this cone. That arising between and slightly below the second and third traces of the lowest whorl may equally

well be looked upon as supra-annular in origin or as having become, speaking phylogenetically, decurrent slightly below the trace of the sporangiophore, with the formation of which it may be associated. Such decurrence of meshes below traces is not uncommon in the cones of certain species (cf. Browne (2), p. 235).

It is in Cone D of this species that the anastomoses characteristic of the strands of the axis at the level of the annulus are most clearly shown. In Cone D four out of six meshes are closed in this region, and three fresh ones arise, one of the first, one of the third order, and one that persists unclosed throughout the whole cone.

In the matter of supra-annular anastomoses Cone E represents a much reduced condition. No fresh meshes arose in this region, and in the immature phase of development represented in Text-fig. 7 none was closed; but the distribution of the phloem indicated that had the metaxylem reached its full development the mesh between the second and third strands would have been closed in the neighbourhood of the insertion of the annulus.

It is characteristic of the cones of *E. debile*, other than Cone D, that some of the meshes originating below the cone, and persisting in it, extend upwards throughout the greater part of the length of the cone. Thus, in Cone A one of the meshes arising above the uppermost vegetative node is only closed at the level of the uppermost whorl of sporangiophores. Cone C affords the most extreme example of unclosed meshes. Only three meshes arise within the cone, two of them unclosed, and one above the annulus, also unclosed. Four of the five parenchymatous meshes found in the internode below the annulus enter the cone, and three of them persist right through it and become confluent round the vascular strand of the acumen. One of these meshes has persisted unclosed not only through the whole cone, but also through the uppermost vegetative node (cf. p. 435).

In the cones of *E. variegatum*, of which serial transverse sections were prepared, no fresh parenchymatous meshes arose in the stele between the level of insertion of the annulus and of the sporangiophores. In Cones A and B none of the parenchymatous meshes originating above the leaf-traces of the last vegetative node was closed in this region. It is possible that the junction of the fourth and fifth strands near the bottom of the reconstruction of the stele of Cone C represents a supra-annular fusion, for the anastomoses of the strands associated with the insertion of the annulus frequently occur somewhat above the attachment of the latter.

It should be noted that in Cones A and C, though there are seven sporangiophores in the basal whorl of the cone, and seven strands below the annulus (these strands having arisen above and between seven leaf-traces), the seven traces entering the sporangiophores are not given off each by a single strand of the internode below, as would have seemed likely in the absence of any clearly supra-annular branchings of the strands. On the

contrary, in Cone A a complex of three strands gives rise to two traces, while two more traces depart from a single strand; and in Cone C three traces depart from a complex of two strands, while another complex of two strands gives off but a single trace. Consequently, in these two cones (though not in Cone B) the traces of about half the sporangiophores of the lowest whorl are anatomically superposed, though somewhat irregularly so, to the corresponding leaf-traces of the last vegetative whorl. At the base



TEXT-FIG. 11. Reconstruction of stele of Cone D of *E. variegatum* from below insertion of annulus to a point above level of first whorl of sporangiophores. Axial xylem black; traces and parenchyma white. $\times 20$.

of Cone D this superposition of the sporangiophore-traces to leaf-traces was also noticeable (cf. Text-fig. 11). It is possible to see in this condition the last indication of the existence in the ancestors of *E. variegatum* of anastomoses marking the former presence of a node, now obsolete, at the insertion of the annulus. Such an interpretation is admittedly very doubtful, and were it not for the numerous anastomoses found in other species in the neighbourhood of the insertion of the

annulus—anastomoses that lead to an irregular superposition of some of the traces of the sporangiophores to certain of the leaf-traces—and for other indications of an obsolete or obsolescent node at this level, the point would not have been mentioned at all.

VII. GENERAL CONSIDERATIONS.

Barratt, in a recent paper, has formulated certain generalizations concerning the cone and annulus of *Equisetum* which seem to me to rest upon an insecure foundation. While refraining from discussing the morphological nature of the sporangiophore, which is too wide a question to be conveniently treated in this paper, I shall, therefore, quote and comment upon some passages from her paper. But before doing this I wish to acknowledge the inadequacy of the description of the xylem of the cone given in my paper on *Equisetum arvense* (Browne (1), p. 666) and quoted in part by Barratt. The quotation as given by Barratt runs as follows: 'She' states (1, p. 666), "In a good many places there are internally to the bands or ring¹ isolated tracheides or little groups of tracheides, usually of small size. . . . Such tracheides or groups of tracheides do not as a rule persist for any considerable distance in a vertical direction; in the internode they occur also internally to the separate strands of xylem." Barratt adds: 'It may be stated quite definitely that these elements belong to the protoxylem, although Browne does not apparently identify

¹ i. e. the present writer.

¹ i. e. of xylem.

them as such, and, as will be shown below, they form a definite system of strands, and constitute the scaffolding on which the whole vascular system of the cone is built up' (Barratt, p. 222). As a matter of fact the little groups of tracheides referred to in my description are undoubtedly protoxylem; but some of the isolated tracheides clearly represent scattered metaxylem elements, slightly internal to the main bands of metaxylem. These single cells are quite irregularly scattered, and do not usually persist for any distance; not being disorganized, as are the tracheides of the protoxylem, they were presumably differentiated later than the latter. As my original description and a similar description of the corresponding tracheides in *E. maximum* (Browne (2), p. 234) not only failed to distinguish between the slightly more internal cells of the metaxylem and the protoxylem, but also failed to recognize the continuity of the strands of protoxylem, they were not only inadequate, but misleading. Barratt's conclusion that the system of protoxylem strands represents the scaffolding on which the whole vascular system of the cone is built up will be discussed later.

'As seen in transverse sections (Text-figs. 20 and 21) the metaxylem is separated from the protoxylem by parenchymatous cells . . .' (Barratt, p. 223). This statement concerning the axis of the cone, taken from Barratt's paper, is too sweeping a generalization. In *E. maximum*, it is true, the internodal protoxylem of the cone seems to be constantly separated



TEXT-FIG. 12. Section of internodal bundle from the axis of the cone of *E. limosum*, showing continuity of some of the metaxylem with the protoxylem. $\times 6,450$.

from the metaxylem by parenchymatous cells. But in the bundle of *E. palustre* figured by Barratt in her Text-fig. 20 certain tracheides abut on the carinal canal, so that the protoxylem was presumably in contact with cells that develop as metaxylem. Metaxylem tracheides abutting on the carinal canals are also shown in figures of the internodes of the cone of *E. palustre* in my first paper on *Equisetum* (Browne (1), Pl. LXV, Fig. 7). In the cone of *E. limosum*, so far as I have been able to observe, the protoxylem of the internodal strand is nearly always in contact with the metaxylem. This relationship is shown in Fig. 8 of Pl. LXV of my first paper on *Equisetum* (Browne (1)), and on a larger scale in Text-fig. 12 of the present paper, representing a transverse section of a small bundle in the internode of the cone of this species. As parenchymatous cells are interspersed in the metaxylem, one or two of the former may be situated between part of the metaxylem and protoxylem, but, as stated

above, the protoxylem seems to be generally in contact with part of the metaxylem. In *E. arvense*, in which the tracts of parenchyma separating the internodal strands of the cone are relatively narrow, the metaxylem develops chiefly in a lateral direction, and is of slight radial extent, so that it is usually separated from the protoxylem by parenchymatous cells. But in this species I have observed that in certain bundles metaxylem tracheides are in contact with the carinal canal for a considerable part of the internode. In *E. hyemale* and *E. sylvaticum* the relations between the protoxylem and the metaxylem in the cone vary between continuity and separation. In *E. sylvaticum* (in my specimens at least) the protoxylem usually abuts on the metaxylem, but in *E. hyemale* either condition seems about equally common. Often in a single section of a bundle the metaxylem abuts on one of the sides of the carinal canal, and is separated from the other by a parenchymatous cell. In *E. giganteum*, in which the metaxylem is of relatively considerable depth, the protoxylem of the cone seems always to be continuous with the metaxylem. Continuity of metaxylem and protoxylem is characteristic also of the internodes of the cone of *E. debile* and *E. variegatum*, though occasional indications of a local separation of protoxylem and metaxylem by one or two parenchymatous cells may be observed.

The above remarks may be summarized by saying that the separation or continuity of metaxylem and protoxylem depends chiefly on the relative radial extent of the former. Where the development of metaxylem is chiefly in a lateral direction (e.g. *E. arvense* and *E. maximum*) separation of metaxylem and protoxylem in the internodes is the result. Where, as in *E. giganteum*, the metaxylem (whether disposed in wide or narrow bundles) is of considerable depth it remains in contact with the protoxylem. Though the internodal strands in the cones of *E. debile* and *E. variegatum* are on a small scale, so that the actual number of tracheides present is small, the radial extent of the metaxylem is considerable compared with its width. The continuity of protoxylem and metaxylem in the internodes of the cones of these species is, therefore, not surprising. In the species in which the protoxylem may be either continuous with or separate from the metaxylem continuity usually occurs when rather more metaxylem is present radially or, more rarely, if the band of metaxylem is slightly incurved. If the vascular system of the cone is a reduced siphonostele it would seem to follow that the separation of protoxylem and metaxylem is a derivative character, resulting from poor development of the metaxylem radially. It should be pointed out that although it is possible that in some cases parenchymatous cells may have been destroyed during the formation of the carinal canals, yet this possibility does not allow of our regarding the metaxylem abutting on the canal as originally separate from the protoxylem; for in many cases the metaxylem at the edge of the canal is

actually continuous with the partially disorganized tracheides projecting into the latter. Moreover, in some specimens of the species in question, continuity of metaxylem and protoxylem may be observed before the destruction of the latter is completed.

'Although such species as *E. arvense* and *E. maximum* show a definite network of strands with short meshes, in *E. palustre*, *E. limosum*, *E. sylvaticum*, the meshes are more irregular—frequently much elongated—stretching for two or more "nodes", and in *E. palustre* even extending the whole length of the cone' (Barratt, p. 226).

As regards this second quotation I find myself unable to agree to the inclusion of *E. maximum* among the species, the stele of the cone of which shows a definite network of short meshes. An examination of Pls. XII and XIII of my paper dealing with the cone of this species will show that the stele possesses a very irregular network of meshes, many of which are long. This is true especially of the larger of the two cones, in which one of the meshes is of the seventh order, and twelve others are of the eighth or higher orders (Browne (2), Pls. XII and XIII). The only reconstruction of part of the network of the cone of *E. maximum* published by Barratt contains too few meshes to afford a reliable basis for generalization. Only thirteen meshes or portions of meshes of any considerable extent are shown; of these, however, only five are of the first order, while two are entering unclosed upon a fourth internode. Again, though in the cones of *E. palustre* the meshes are, on an average, much longer than those of *E. arvense*, they can hardly be said to extend nearly the whole length of the cone, at any rate on the evidence published, though of course this may be so in specimens with a reduced vascular system.¹ Such a description would, however, apply to certain of the meshes of the cones of *E. limosum*.

After concluding that the existing structure in vegetative and reproductive axes has been derived from a siphonostele, Barratt proceeds to deal with the problem of the determining factor associated with the distribution of the parenchymatous tracts. Of this question she writes: 'Can it be met by an application of the conception of foliar gaps which is associated with the study of filicinean anatomy? Browne² has attempted such an explanation in her papers dealing with the cones of several species of *Equisetum*. She homologizes the parenchymatous meshes with foliar gaps, associating them with sporangiophores, and considers that these organs are modified whole foliar structures. . . .

'Even if the sporangiophores were foliar structures, the anatomy does not favour the view that the meshes are foliar gaps. Jeffrey has pointed

¹ In the case of the reconstruction published by me (Browne 1), Text-fig. 4' of the stele of the variety *polytachion* of *E. palustre* the meshes may be said to extend nearly the whole length of the cone. This is, however, not due to their height, but to the reduction of the whorls of these cones, borne on lateral branches, to four.

² i. e. the present writer.

out, and it is abundantly confirmed in this investigation, that the gap does not, except in a few cases, occur immediately above the point of departure of the trace; and most frequently shows no relation to it.' (Barratt, p. 231.)

Barratt's own view on the determining factor in the distribution of parenchymatous meshes is given as follows on p. 227 of her paper: 'It is suggested that the determining factor in the relative development of the metaxylem, and hence of the meshes, is primarily a mechanical one. It is significant that the species with large and heavy cones have more abundant xylem and more regularly developed network.'

The first point that I should like to emphasize in connexion with the above quotations is that I have never regarded the parenchymatous tracts of the cone as foliar gaps, or even as gaps left by the traces of the sporangio-phores. In my first paper treating of the cone of *Equisetum* I especially pointed out that the parenchymatous meshes of the axis of the cone, like those of the vegetative axis, do not, except at the reduced or immature apex of the cone, originate immediately above the traces, and that they are not foliar gaps as defined by Jeffrey (Browne (1), p. 698). I proceeded to compare these meshes with the tracts of parenchyma that originate some way above the traces of certain Osmundaceae, and pointed out that these Osmundaceous parenchymatous tracts were not, when they originated some way above the trace, foliar gaps. I held that the structure now found in the axis of the cone of *Equisetum* was derived from a siphonostele, a view with which Barratt has expressed agreement. I further suggested that in the phylogeny the parenchymatous meshes probably arose in the internodal region vertically above traces—not a surprising position when we remember that the current of water, some of it being deflected into the sporangio-phores, would be diminished above the departure of the traces. But I especially added that the cases in which the mesh arose closer to the trace were probably instances of the reduction of xylem (Browne (1), p. 699). In fact, it was to emphasize the point that the parenchymatous tracts of the cone were *not* foliar gaps that I distinguished them by the term mesh.

Barratt's statement that the meshes of the cone *most frequently* show no relation to the departure of the traces seems to me unjustified. An examination of the reconstructions of complete steles of the cones of *E. arvense*, *E. palustre*, *E. limosum* (Browne (1), Text-figs. 1, 3, 5, and 6), *E. maximum* (Browne (2), Pls. XII and XIII), *E. hyemale*, *E. giganteum* (Browne (3), Text-figs. 2, 3, 4, 5, 6, and 7), *E. sylvaticum* and *E. variegatum* (Text-figs. 1, 2, 8, 9, and 10 of the present paper) will show that in by far the greater number of cases meshes originating within the cone *do* show a relation to the point of departure of a trace; for, in the great majority of cases, these meshes arise vertically above the departure of a trace of the node below, though at a varying height above this node. In *E. debile* (Text-figs. 3-7 of the present paper) few meshes originate within the cone

itself. In Cones A, C, and E much of the parenchyma found in the steles consists of meshes that arose above the last whorl of leaves and persist well into the cone. In Cone E, indeed, only two fresh meshes originate within the cone proper—i. e. definitely above the basal whorl of sporangiophores.¹ But even in the cones of *E. debile*, where the vascular system is so reduced, never less and sometimes more than half the parenchymatous meshes originating within the cone itself arise vertically above traces of the node below.² Taking into consideration the reconstructions of the steles in all the nine species studied, the proportion of meshes that arise vertically above traces is very large. Moreover, the fact that this relationship is much more prevalent in species with more metaxylem and shorter meshes is surely an argument in favour of the primitiveness of that position for those who, like Barratt and myself, look upon the primitive type of vascular structure in the cone as a siphonostele. Though it is suggested that the primitive form of mesh was probably one of the first order, originating at a point superposed to and some way above a trace of the whorl below, it is not, of course, claimed that in any one cone on the line of descent of *Equisetum* a parenchymatous mesh of the first order was found above every trace. Indeed, this was almost certainly not so, since some of the existing species of *Equisetum* retain in places wide internodal sweeps of xylem, apparently a vestige of the former siphonostele, although all the existing species of the genus possess some meshes of orders higher than the first.

The only argument given in support of the suggestion that the factor governing the distribution of the meshes of parenchyma may be a mechanical one is that 'the species with large and heavy cones have more abundant xylem and more regularly developed network' (Barratt, p. 227). It has already been pointed out that in the cones of *E. maximum*, the largest of the genus, there is a very irregularly developed network and relatively little xylem. Of course, in a cone of this species which, like Cone A of my paper on *E. maximum*, consists of over six hundred sporangiophores there are actually a large number of tracheides, although the xylem is poorly developed relatively to the parenchyma.³ I have previously suggested that while the length of the cones seems to have little effect on the height of the meshes the width of the stele has a bearing on the closure or persistence of the meshes through the node. I wrote: 'But if the reduction in width of the stele did not keep pace with the reduction of the xylem at the

¹ In estimating the nature and incidence of the parenchymatous meshes of this cone and of Cone B of the same species it must be remembered that the broken line enclosing a dotted surface marks the extent to which the differentiation of metaxylem would presumably have attained had the cone been mature.

² An indirect confirmation of the view that the cones of this species are amongst the most reduced of those studied is afforded by the fact that in the branches bearing them the ring of xylem at the last node may be incomplete, a condition not hitherto recorded for other normal nodes.

³ This cone was not exceptionally large. It was about three inches long, and I have seen cones fully four and a half inches in length.

Neighbourhood of the node, the direct and immediate effect would be for some of the meshes to persist into the internode above. I think that this is the explanation of the greater reduction of the vascular system in the cones of the species of *Equisetum* that have relatively wide steles.' I then pointed out that of the four species so far studied the two with the widest steles, *E. maximum* and *E. limosum*, had the most reduced vascular system, while within the limits of the single species, where the examples studied had steles showing a marked difference in diameter, the more irregular and reduced vascular network was always found in the specimens with wider steles. I added: 'Of course the width of the stele is not the only, nor the principal factor causing reduction of the vascular system; it is, for instance, characteristic of *E. arvense* to have cones containing more axial xylem, both actually and relatively to their size, than those of *E. palustre*, though the steles of the former species are usually much wider than those of the latter' (Browne (2), pp. 259-60).

The generalization contained in the former quotation should, I think, be modified by the intercalation after the words 'at the neighbourhood of the node' of the following passage: 'or with the reduction in number of the members of the whorls.' This qualification is necessary to cover cases, like that of *E. debile*, in which the whorls of the cone consist of few members, so that though the diameter of the stele is actually small the narrow internodal strands are separated by relatively wide parenchymatous meshes. We naturally find that in the species in which the meshes are wide compared with the internodal strands (*E. maximum*, *E. limosum*, *E. variegatum*, and *E. debile*) they are less regularly closed than in the species with narrow meshes relatively to the strands (*E. arvense* and *E. palustre*), since the junction of a strand with its neighbour or neighbours can here be effected by the differentiation of comparatively little xylem additional to that present in the internode. *E. sylvaticum* and *E. giganteum* occupy a middle position between the types with relatively wide and relatively narrow meshes. *E. hyemale*, with its wide stele and narrow internodal strands separated by wide tracts of parenchyma, is an exception to this generalization; for it shows a large number of meshes, many of them of the first or low orders. In the cone of this species the difficulty of anastomosis between widely separated narrow strands has been, to a large extent, met by the oblique course of the tracheides on each side of the base of the meshes. Speaking phylogenetically, we should say that in spite of the reduction of the xylem the connexion of the nodes of the vascular strands has been in many cases maintained by the oblique course of the halves of the forking strands. The divergence of these branches of strands frequently results in their fusion with the neighbouring fork of a strand (as in the vegetative axis of *Equisetum* generally), or, if the neighbouring strand be unforked, with the latter itself. In either case this causes the closure of a mesh slightly above the level of departure of the trace.

That the course of the protoxylem should be irregular is only what we should expect to find in a stele undergoing reduction. In so far as the elements of the protoxylem are the first tracheides to be differentiated in the ontogenetic building up of the stele I think that the protoxylem system may well be compared, as it is by Barratt, to a scaffolding. She, however, also states that the whole vascular system of the cone is built up on this scaffolding, and claims that the protoxylem strands determine the main features of the vascular system (Barratt, pp. 222 and 225). If it were so the comparison would be an unfortunate one, for a building is constructed by means of a scaffolding, but not on a scaffolding, a temporary structure that disappears. I do not, of course, mean that the protoxylem is merely a temporary scaffolding, for it remains functional though some of its elements are destroyed, and undoubtedly plays an important part, since it constitutes the first and by far the principal source of the vascular supply of the sporangiophores. But that the protoxylem strands should alone determine the main features of the vascular supply is hardly tenable; for the development of a parenchymatous mesh above a trace, though occurring later in the ontogeny, must be associated with the forking or swerving or the dying out of the strand of protoxylem, unless the trace is one of those borne on a short lateral stalk of protoxylem (see below and p. 454). Both the dying out of protoxylem below a mesh and its swerving to one side of the latter seem to be derivative conditions, associated with less vigorous development of the protoxylem. In the reconstructions of the steles of Cone B of *E. sylvaticum* (Text-fig. 2) and of Cone A of *E. debile* (Text-fig. 4) the course of the protoxylem is indicated by a broken white line. Where the growth in thickness has caused the destruction of the protoxylem and its replacement by a lacuna the width of the latter is shown by a dotted surface. The original elements did not, of course, occupy so wide a space as the lacuna. It will be seen that, especially in *E. sylvaticum*, in which the vascular system is better developed, forking of the protoxylem occurs in many cases below a mesh. When the protoxylem forks such forking obviously occurs before the appearance of a mesh causes the division of the metaxylem, and it not infrequently takes place a little below the departure of the trace. This is by no means surprising, as when a stele or meristele is about to fork the division of its protoxylem often occurs somewhat prematurely. When the division of the protoxylem occurs thus early the trace often appears as though terminating a short median prolongation of the protoxylem between the two forks of the latter. In the case of some of the protoxylem strands that pass unforked through the node the trace also appears in the reconstructions to be seated on a short stalk, though in this case on a lateral one. In such cases the protoxylem of the appendage departs from that of the axis a little below the exit of the trace from the stele, and, as the sporangiophore to which the trace is destined does not

lie opposite the axial strand, the vascular supply as it passes gradually upwards and outwards through the bundle curves towards the sporangiphore and comes to lie on a different radius from the parent strand of the axis. Thus it is shown as a separate broken line in the reconstruction. The cases in which the axial protoxylem is shown in the diagrams as passing straight and uninterruptedly upwards above the traces are of three sorts. Firstly, the protoxylem of the trace may pass out horizontally, and then the axial protoxylem does not branch below the trace; or the protoxylem of the latter may enter a sporangiphore on the same radius as the axial strand, and so never became apparent as a separate branch in the reconstruction; or, lastly, such a trace may enter a sporangiphore to one side of the axial protoxylem, but the curvature of the trace may occur in the cortex (cf. Browne (2), p. 261, on the twisting of the traces in the cortex of *E. maximum*).

That the course of the protoxylem as well as the distribution of the metaxylem has been affected by reduction seems to me confirmed by the fact that in Cone B of *E. sylvaticum* the protoxylem entering the ninth sporangiphore of the eighth whorl, and that of the fourth sporangiphore of the uppermost whorl, are not continuous with the rest of the protoxylem of the cone. The protoxylem of the axial strand giving rise to the ninth trace of the eighth whorl forms a particularly distinct canal, abutting at its lower end on very definite metaxylem tracheides. This means that in the ontogeny the cells just below the lower end of the axial protoxylem had reached a considerable size before they were differentiated as tracheides, and that such differentiation occurred later than the differentiation of the tracheides of the protoxylem immediately above them.

We may perhaps recognize in the incomplete fusion of certain protoxylems, which is one of the irregularities of the protoxylem 'system', a phenomenon associated with prematurely divided traces. Such approximation of protoxylems, not leading to their junction, as is found, for example, at the point of departure of the eighth trace of the fourth whorl of Cone B of *E. sylvaticum*, causes the trace to originate as separate halves. This failure of two protoxylems to fuse into a single one is, it may be noted, associated with a failure of one of the strands to branch at the node below, and the persistence of the mesh through this lower node to a point just below the next node. In this case the metaxylem of the two strands, which fuses just before the fourth node, clearly reproduces the more primitive condition lost by the protoxylem.

SUMMARY.

1. The vascular system of the cone of *E. sylvaticum* resembles on a smaller scale that of the cone of *E. maximum*; relatively to its size it is better developed.

2. The vascular system of the cone of *E. debile* is much reduced and forms a loose irregular network. Frequently numerous parenchymatous meshes originating below the cone persist for a considerable distance into the cone, some of them even traversing the whole cone.

3. The stele of the cone of *E. variegatum* is also of a somewhat reduced type.

4. On grounds of comparative anatomy the separation of protoxylem and metaxylem in the internodes of the cones of certain species of *Equisetum*, a condition most marked in *E. maximum*, is regarded as a derivative character, due chiefly to reduction of the radial extent of the metaxylem.

5. It is concluded that a comparative study confirms the view already put forward that parenchymatous meshes of the cone probably first arose, in the phylogeny, at points vertically above the departure of the traces of sporangiophores, though at a certain height above this level. They were, therefore, not true gaps, as defined by Jeffrey. In the cases in which the meshes arise very close above the departure of the trace, this approximation is probably due to reduction of xylem during the phylogeny. The primitive system was probably siphonostelic.

6. The following points in the anatomy of *E. sylvaticum* support the view that the insertion of the annulus marks the position of a vestigial node: (a) the numerous anastomoses of the axial strands at this level; (b) indications of anastomosis of the protoxylem at the same level; (c) the presence in the axis opposite the annulus of Cone B of an abortive trace that never becomes free; (d) the presence in this same cone at the level of insertion of the annulus of tracheides somewhat resembling those of the nodal xylem of the vegetative axis; (e) the occurrence, according to Milde, of branches below the annulus.

7. In one of the cones of *E. debile* four small groups of vascular cells were observed in the parenchyma of the annulus. Though they are unconnected with cells of the axial bundle it is possible that these vascular cells represent vestiges of the traces of the annular node. In this species, however, and still more in *E. variegatum*, there are but few indications of the former presence of a node at the insertion of the annulus.

8. In some specimens of *E. debile* the ring of nodal or (supranodal) xylem is not complete at the level of the uppermost whorl of leaves.

My thanks are due to Professor F. W. Oliver, F.R.S., in whose laboratory the present investigations were carried out, not only for his continued help and encouragement, but also for material of *E. variegatum*. Major T. G. Hill, A.R.C.S., most kindly gave me the benefit of his advice on certain points of technique. I also wish to thank Dr. Shriv Yam Kashyap, who sent me from India a most generous supply of the cones of *E. debile*, of all sizes and ages; and Miss Agnes Fry, who supplied me with cones of *E. sylvaticum*.

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EXPLANATION OF PLATE XXI.

Illustrating Lady Isabel Browne's paper on the Anatomy of the Cone and Stem of *Equisetum*.

Fig. 1. Transverse section of part of the axis of Cone B of *E. sylvaticum* a little above the insertion of the annulus. Note relatively numerous tracheides, some rather wide. $\times 65$.

Fig. 2. Transverse section of part of the axis of Cone B, taken a little below Fig. 1, but still slightly above the insertion of the annulus. Note the forking of some of the protoxylem canals; other bundles still retain a single, relatively large carinal canal, such as characterizes all the bundles in the lower part of the internode. $\times 65$.

Fig. 3. Transverse section through part of the axis and annulus of Cone B of *E. debile*, showing a longitudinal view of one of the tracheides of a small free annular bundle. The photograph is taken at the level at which the annular vascular tissue approaches closest to the axial stele. $\times 150$.

Fig. 4. Transverse section of the stele of the cone of *E. variegatum* near the level of a node. As the sporangiophores are not inserted absolutely at the same level the section only passes through the insertion of two of the traces. $\times 125$.

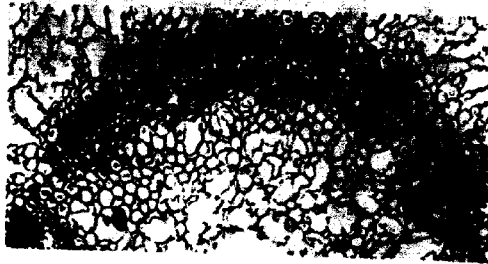
Fig. 5. Transverse internodal section of the stele of *E. variegatum*. One of the bundles on the reader's left shows the relatively large deeply seated tracheides of the metaxylem particularly clearly. $\times 125$.

Fig. 6. Transverse section of the axis of *E. debile* between the last whorl of leaves and the insertion of the annulus. Note that the bundles are situated opposite the grooves of the stem. $\times 30$.

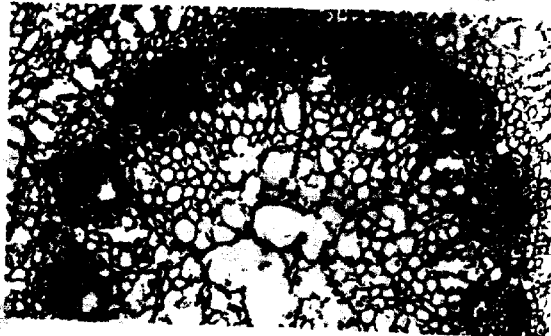
Fig. 7. Transverse section of part of the stele of an internode of the cone of *E. debile*. Note the difference in the size of the bundles and the different degree of destruction of the protoxylem. Other points to be noted are the definite sheaths surrounding the bundles, the wide parenchymatous meshes, and the relatively large size of some of the deeper seated metaxylem tracheides. $\times 65$.

Fig. 8. Transverse section through a node of the stele of *E. variegatum*. This specimen was exceptionally large and the xylem unusually well developed. $\times 125$.

Fig. 9. Upper surface of the annulus of Cone B of *E. debile*, showing two stomata. $\times 500$.



1.



2.



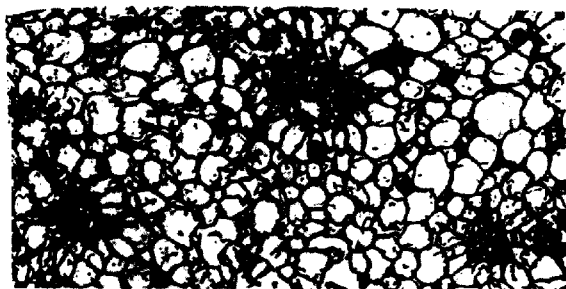
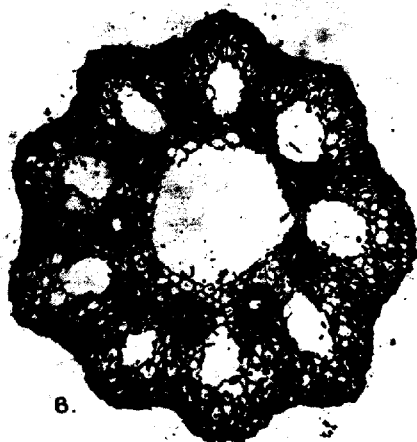
3.



4.



5.



NOTES.

NOTE ON THE OCCURRENCE OF SEQUOIA IN THE HEADON BEDS OF HORDWELL, HANTS.—While looking for seeds in the Leaf Bed of the Lower Headon series, Hordle (Hordwell), Hants, good specimens of *Athrotaxis Coultsiae*, so called by Starkie Gardner, have become available for study.

The material, which was in an uncrushed condition, yielded twigs and leaves, cones and cone-scales, and abundant seeds, and the discovery of one cone which still contained many seeds, and of another which was still attached to its twig, furnished indisputable evidence that the different organs found really belonged to one and the same species.

As the result of careful microscopic examination of leaves and stomata, of seeds, and of cones and cone-scales, the fossil can now be referred with confidence to the genus *Sequoia*.

Great interest was aroused by Starkie Gardner's reference of this Hordle fossil to the endemic Tasmanian genus *Athrotaxis*, for he stated that it was the same species as *Sequoia Coultsiae*, Heer, from the Hempstead Beds; at the same time he threw doubt upon other records of *Sequoia* from Eocene and Oligocene horizons.

Until 1910 no attempt was made to clear up the uncertainty thus occasioned, but in that year Mr. and Mrs. Clement Reid, after thorough investigation, confirmed Heer's determination of the Bovey Tracy species as *Sequoia*.

Now that material from Starkie Gardner's original locality for *Athrotaxis* has also proved to be *Sequoia*, it seems desirable to state the fact, pending a full account of the whole work for which there is not at present space.

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PHLOEM NECROSIS (BROWN BAST DISEASE) IN HEVEA BRASILIENSIS.¹—The disease known as brown bast is prevalent in all tropical countries where *Hevea brasiliensis* is grown on a large scale for commercial purposes, especially in plantations where tapping is in progress, and it is regarded at the present time as the most serious malady to which the Pará rubber tree is liable.

The external symptoms of brown bast are manifest in the form of longitudinal irregular cracks in the bark and nodular swellings (burs), which are usually confined to the basal portions of the trunk and the larger lateral roots. The presence of

¹ Farmer, J. B., and Horne, A. S.: On Brown Bast and its Immediate Cause. The India-rubber Journal, vol. lxi, No. 25, June 18, 1921, p. 25.

[Annals of Botany, Vol. XXXV, No. CXXXIX, July, 1921.]

brown bast in a tree may be suspected when it ceases to yield latex in response to tapping, or when the amount of latex obtained at each tapping shows considerable diminution, in which case an uneven exodus of the latex can be detected by an inspection of the cut surface immediately after the tapping operation has been performed.

Some description of the anatomy of the burrs has been given recently by Sanderson and Sutcliffe,¹ who attribute them to meristematic activity of the parenchymatous cells which abut on the diseased laticiferous vessels: the new tissue cells may be converted into stone cells or differentiated into woody elements.

Rands,² in a preliminary note, states that the tissues forming the bark of *Hevea brasiliensis*, when wounded, produce a gum which blocks up the intercellular spaces and causes a brownish discoloration of the tissue in the neighbourhood of the wound. Bobiloff³ states that as brown bast develops the anatomical changes to be observed in the bark include the formation of a brown degenerate substance in the intercellular spaces and middle lamellae of the cells.

Whilst studying transverse sections of the bark taken from certain trees affected with brown bast and from trees in which the presence of the malady was suspected, using material obtained by Professor J. B. Farmer from British North Borneo, numerous minute golden yellow spots of irregular outline were observed in the phloem, in the region extending from the neighbourhood of the cambium outwards. With a higher magnification the outlines of the coloured granular areas sometimes resembled those of intercellular spaces, but were distinguishable from spaces owing to their intersection by one or more waved partitions staining more or less distinctly with ruthenium red. After a detailed study and careful comparison with the phloem in normal bark, these golden areas were found to be the sections of necrotic sieve-tubes which no longer showed a reaction for callus with soluble blue; and the waved outlines in many cases could be interpreted as transverse sections of the large vertical sieve-plates which form the dominant feature of the phloem of *Hevea* when viewed in longitudinal section.

In order to be certain that the disease affecting the Borneo material was actually brown bast, reference was made to authentic material from the Federated Malay States, with the result that similar pathological characteristics were discovered in the tissues of the Malayan specimens.

In the younger phloem the disease is confined to the sieve-tubes, but in the middle phloem region the discoloured areas are larger, owing to the fact that other cells—phloem parenchyma, medullary ray cells, laticiferous vessels—have become involved in the local tissue degeneration.

Incipient stages in the process of burr formation have been observed. The wound cambiums arise in proximity to the diseased laticiferous vessels and often completely encircle small groups of vessels. As a result of the activities of the wound

¹ Sanderson, A. R., and Sutcliffe, H.: Brown Bast. Published by the Rubber Growers' Association, May, 1921.

² Rands: De bruine binnenbastziekte van *Hevea brasiliensis* (Voorloopige mededeeling). Archief voor de Rubbercultuur in Ned. Indie, 1919, April, Jaarg. III, p. 156.

³ Bobiloff, W.: Over de oorzaak der bruine binnenbastziekte van *Hevea brasiliensis*. Bot. Mai, Jaarg. III, p. 172.

cambium, diseased groups of cells, including laticiferous vessels, become enclosed in a 'pocket' of stone cells. The number of meristematic zones will depend on the number of rows of laticiferous vessels affected and their disposition in the bark.

According to the evidence collected by Bateson, Sanderson and Sutcliffe (Malaya); Rands and Bobiloff (Java), the brown bast disease is attributable to physiological causes. Keuchenius,¹ who has isolated bacteria from the bark of trees affected with brown bast, has not succeeded in causing the disease as a result of inoculating healthy trees with the organism obtained. Owing to the occurrence of widely distributed sieve-tube degeneration in the tissues affected with incipient brown bast, the disease is analogous to the cases of phloem necrosis described for the potato by Quanjer² and for Liberian coffee by Stakel.³ The special anatomical features described by Sanderson and Sutcliffe would appear to be a secondary development.

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¹ Keuchenius, P. E.: Onderzoekingen over bruine bastziekte. *Archief voor de Rubbercultuur in Ned. Indie*, 1920, Feb., Jaarg. iv, p. 1.

² Quanjer, H. M.: Die Nekrose des Phloems der Kartoffelpflanze als Ursache der Blattrollkrankheit. *Mededeelingen, Rijks Hooge Land-, Tuin- en Boschbouwschool*, Deel vi, 1913.

³ Stakel: Phloemnecrose of Liberian Coffee. *Bull. Dept. van de Landbouw in Surinam*.

Observations on the Anatomy of Teratological Seedlings.

IV. Further Studies on the Anatomy of Atypical Seedlings of *Impatiens Roylei*, Walp.

BY

H. S. HOLDEN, M.Sc., F.L.S.,

AND

MARGARET E. DANIELS, B.Sc.

With ninety-seven Figures in the Text.

THE present paper is the outcome of an attempt to determine the relationships of abnormal seedlings of two types occurring in *Impatiens Roylei*, the anatomy of which has been described in a previous issue of this Journal (18). In the normal seedling each cotyledon possesses a median 'double' bundle with independent laterals, and shows tetrarch symmetry in both hypocotyl and root. The tetrarch condition is reflected in the constant presence of a whorl of four lateral roots at the junction of the hypocotyl and tap-root. In the abnormal seedlings one type¹ described exhibits the characteristic phenomena associated with syncotily, cotyledonary fusion being followed either by a precocious fusion of the laterals on the symphysis side with the midrib, or by their partial or complete suppression. A triarch symmetry is thus produced and is accompanied by other related features, notably the development of three members in the root-whorl and the elimination of the leaf on the symphysis side at the first epicotyledonary node. The second type² apparently possesses a single cotyledon only, and gives rise to a diarch condition in the hypocotyl, this being reflected both in the root-whorl and tap-root. One of the two poles in the hypocotyl is a direct downward continuation of the single median 'double' bundle: the other results from the fusion of the lateral strands on either flank. Only one axillary cotyledonary bud is present, in contrast to the two occurring in the first type. The two types approach each other, as far as

¹ Referred to subsequently in this paper as 'Type 1'.

² Referred to subsequently in this paper as 'Type 2'.

previous investigation has shown, solely in the epicotyledonary modifications, which are strikingly similar. It will be obvious that the mode of origin of seedlings of the second type raises a point of some theoretical interest. If they are produced by cotyledonary suppression, then syncotylly and heterocotylly coexist in the same species: if they represent the ultimate phase in a syncotylous series, then the two cotyledons exhibit so perfect a dovetailing of parts that all traces of double origin are lost.

It was realized that suitable material for the determination of this point would be scarce, and in all some 118 seedlings have been microtomed without yielding absolutely conclusive evidence. As was inevitable, however, collateral problems of interest have arisen, and these have also been followed up and the results included in the present paper.

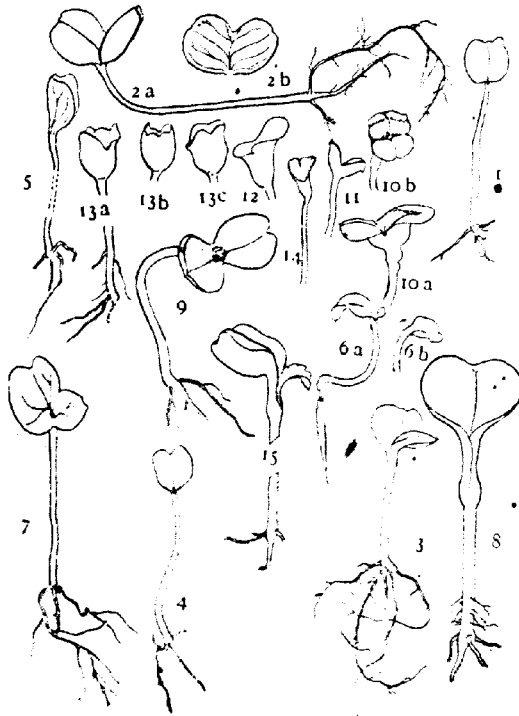
The direct evidence which bears upon the origin of the seedlings of the second type falls under two heads, namely:

(a) The behaviour of the 'double' bundles in certain undoubted syncotyls.

(b) The variations in the cotyledonary axillary buds.

Two clear cases of complete midrib-fusion were obtained (Seedlings A and B), and these will be described in detail. In 'Seedling A' (Fig. 1), in which a closely syncotylous condition obtains, the respective median strands travel down the compound lamina in close proximity and, whilst still separate, develop 'double' bundle structure (Fig. 16). They are inclined slightly to each other, and for some distance subsequent to their union two mesarch protoxylem groups are evident in the common metaxylem, with a phloem group on either flank and one in the plane of junction (Fig. 17). The fate of the median phloem is somewhat curious, the major part passing over to unite with one of the lateral groups, while the remnant dies out. The compound bundle loses all traces of its double origin, and forms a single pole both in the hypocotyl and root. The marginal strand and the lateral of one side fuse and, near the apex of the hypocotyl, unite with the marginal strand only from the opposite side, the composite bundle becoming exarch and constituting a second pole (Fig. 18). The protoxylem of the lateral which remains independent persists in an endarch position for a short distance and then disappears, being followed at a lower level by the metaxylem, so that below this point the hypocotyl is diarch for a period (Fig. 19). The two xylem poles associated with the cotyledonary strands are augmented by two which rise *de novo* in the plane at right angles to them, and a tetrarch condition is thus produced, giving rise to four lateral rootlets in the root-whorl, and below these to a tetrarch tap-root (Fig. 20). In 'Seedling B' the march of events is somewhat different, but is also of interest. The seedling (Figs. 2a, 2b) is an obvious syncotyl, but in the bottom third of the compound lamina the midribs, which are represented by collateral bundles at this stage, approach each other at an angle of almost

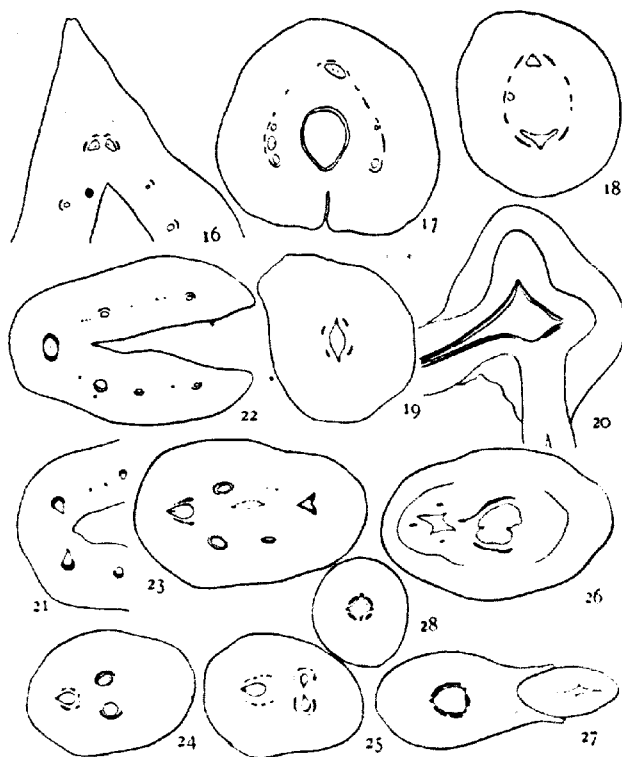
90° from their normal position, the phloems lying farthest from the line of cotyledonary fusion (Fig. 21). The two bundles unite by their protoxylems and form a mesarch mass almost surrounded by phloem (Fig. 22). At the apex of the hypocotyl the protoxylem is tending to assume an exarch position, and the phloem, which behaves in a curiously anomalous manner



FIGS. 1-15. Fig. 1. 'Seedling A.' A syncotyl combining tetrarchy at the base of the hypocotyl with extremely intimate syncotily. Figs. 2a, 2b. 'Seedling B.' A syncotyl in which the hypocotyl is diarch; Fig. 2b shows the venation of the compound lamina. Figs. 3, 4. Syncotyls showing tetrarchy. Fig. 5. A syncotyl showing a triarch hypocotyl; the tap-root was pentarch. Figs. 6a, 6b. Seedling with markedly unequal cotyledons. Fig. 7. A syncotyl with unequal cotyledons. Fig. 8. Large 'Type 1' seedling in which the epicotyl has not developed. Figs. 9, 10a, 10b. Salver-shaped amphisyncotyls, 'Seedlings C and D' respectively. Figs. 11-14. Goblet-shaped amphisyncotyls. Fig. 15. 'Seedling H.'

throughout, is creeping round the inner margin of the composite bundle (Fig. 23). At a slightly lower level it forms a U-shaped system as seen in transverse section, with a gap where the protoxylem is situated (Fig. 24). Meanwhile the marginal bundles of each cotyledon fuse and, after a brief downward course, end blindly. The lateral bundles, which also show

anomalies in their phloem arrangement, after receiving a number of accessory strands, unite in the uppermost third of the hypocotyl (Fig. 25) and, the protoxylem becoming exarch, a diarch condition is produced which persists throughout the hypocotyl and results in the giving off of two stout lateral roots at the point of junction with the tap-root (Fig. 26). Immediately



FIGS. 16-28. Figs. 16-20 'Seedling A', Figs. 21-28 'Seedling B'. Both show a fusion of the cotylar midribs, these behaving in transition like a single normal midrib. These and all subsequent figures are camera lucida drawings reduced to scale.

below the point of origin of the lateral roots the diarch condition is still evident, in spite of a considerable amount of secondary thickening which has produced a tracheide complex with a continuous sheath of phloem. At a slightly lower level the protoxylem, which is continuous with that of the fused laterals, flattens out and bifurcates, the phloem sheath at the same time breaking into four parts (Fig. 27). Three of the gaps in the phloem are opposite the protoxylems derived from the median and lateral bundles

respectively. The fourth gap heralds the formation of a new protoxylem pole which arises laterally and quite independently of the others. It is evident that the tetrarch condition of the main root which characterizes both these seedlings is evolved in a manner which differs fundamentally from that of the normal type. The question of the origin of the root-poles in the abnormal seedlings as a whole will be considered in detail at a later stage, but the chief point for immediate consideration is that provided by the behaviour of the united median strands, which lose all trace of their double origin and act in a manner precisely similar to a normal midrib, forming between them a single root-pole. Dr. E. N. Miles Thomas (31) has recorded a somewhat similar case in an abnormal specimen of *Anemone pulsatilla*, the two fused midribs of which are continuous with one root-pole of a diarch root, the other being in the plane of the first plumular leaf. The same author, jointly with Miss Davey (32), has investigated the seedling anatomy of a number of Pseudo-Monocotyledons, though their results have been up to the present only published in abstract. Material of *Anemone apennina* and *Ranunculus Ficaria*, in which the single member is more or less bifid, and of *Conopodium denudatum*, in which it is undivided, was among that examined. In all the root is diarch, the xylem plane passing through the centre of the first plumular leaf, a feature which they share with the seedling of *Anemone pulsatilla* described above, and in which they differ from their normal dicotyledonous relatives where the diarch plate is at right angles to this plane. In *Ranunculus Ficaria* root structure is initiated below the cotyledonary node, the vascular supply of the first epicotyledonary leaf apparently contributing largely to one root-pole. In *Conopodium denudatum* and *Anemone apennina*, on the other hand, root structure is present from the lower half of the cotyledonary 'petiole' downwards, and inferentially, only the cotyledonary vascular strands connect directly with the root-poles. It will be recognized that the two latter forms bear a fairly close resemblance to the 'Type 2' seedlings of *Impatiens Roylii* described here, whilst *Ranunculus Ficaria* is nearer in type to certain Monocotyledons such as *Allium* and *Triglochin*.

Other syncotylous specimens of *I. Roylii* showing a linking of the adjacent half-phloems of the 'double' bundle and occasionally forms with a temporary linking of the xylem also were met with, but these showed the usual triarchy in the hypocotyl with its accompanying three lateral roots.

It seems reasonable from the evidence yielded by Seedlings A and B

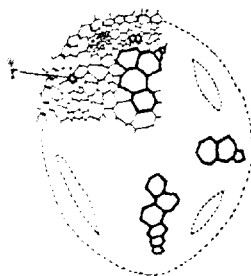
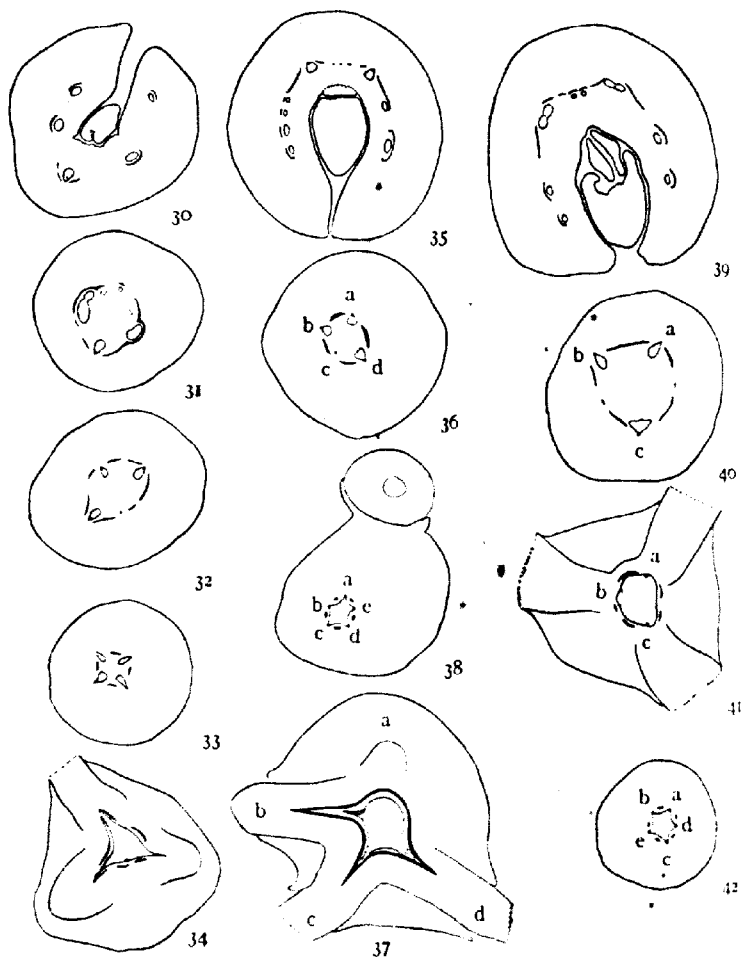


FIG. 29. Transverse section of stele, showing the origin of a root-pole, *r. de novo*.

and from that provided by other species to suppose that extremely intimate syncotyl may lead to the suppression of all evidence of double origin in the composite petiole, and, if carried farther, in the cotyledon lamina also. The evidence yielded by the behaviour of cotyledonary buds, though different in character, is of no less interest in this connexion. The buds in the axils of the cotyledons are not developed very early, and in much of the material studied they are either not evident or are doubtfully represented by small groups of meristem. In the older syncotylous seedlings an excellent series of forms showing progressively closer fusion of the buds has been secured. In the simplest type the double origin of the bud is very evident, as it consists of a tangentially elongated ovoid mass of meristem which bifurcates near the apex (Fig. 82). Other stages show a rather massive bud with apical splitting, and others again show what to all appearance is a single normal bud (Fig. 35). In addition to this series a solitary specimen was discovered among the 'Type 2' seedlings which showed an axillary bud which was quite evidently of double origin. There was thus in this type of seedling indications of the double nature of the cotyledonary bud, whilst the 'Type 1' series shows that, as is the case with the midribs, a fusion so intimate may occur that all evidence of dual origin is suppressed. When these facts are considered, together with the striking similarity in the modifications of the epicotyl, the view that the whole of the abnormal seedlings must be regarded as syncotylous in origin is materially strengthened. Further collateral evidence is provided by a similarity in the behaviour of the vascular strands during their passage from the cotyledon to the root in the two types of seedling, and also by certain new features in the transition region which exhibit a close parallelism. Considerable numbers of modified seedlings were obtained which, although their appearance would suggest a triarch and diarch condition of the hypocotyl, nevertheless possessed a root-whorl with four members (Figs. 3, 4), and therefore presumably four xylem poles. An investigation of these revealed the fact that the additional poles may arise in one of two ways. One has already been indicated in the previous paper dealing with these seedlings (18), and is due to the combination of two features, namely, the growth in size and importance of the marginal as distinct from the lateral strands, and the remaining independent of the latter so that the marginals alone contribute to the root-pole opposite that formed by the midrib. The independent laterals are often continued into the hypocotyl as endarch strands, which usually soon lose their protoxylem and ultimately die out. Prior to this they may become mesarch and occasionally, near the base of the hypocotyl, exarch, though the position of the protoxylem, where persistent, is inconstant. It is from this type of persistent lateral strand that a new member of the lateral root-whorl and subsequently a root-pole originate. Neither the fact of the protoxylem remaining endarch nor its disappearance at a lower level seem to affect

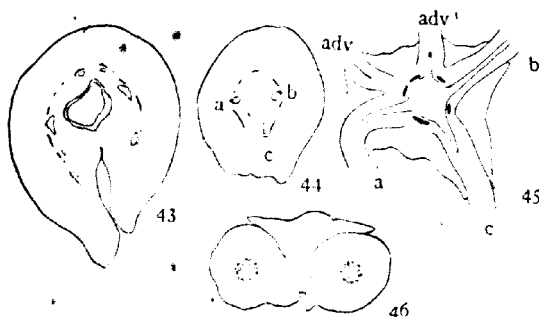
this, although where the protoxylem is lost high up in the hypocotyl no corresponding lateral root or root-pole is usually formed. The formation of one additional root-pole in this manner is a fairly common feature of both types of seedling, and results in either a triarch or a tetrarch condition. It is due in such cases to the asymmetrical persistence of one lateral only. In the previous paper (18) dealing with these seedlings a comparison of the independent laterals with the 'Zwischenstränge', first described by Dodel (11) and later by Compton (5, 6), was made and the differences between the two types noted. This difference has received emphasis by the discovery of strands showing much closer affinities with true 'Zwischenstränge' in the series of seedlings now described. These strands, each of which normally gives rise to a new member of the lateral root-whorl and subsequently to a root-pole, usually originate in the basal third of the hypocotyl, and at first are represented by an isolated tracheide (Fig. 29) lying in the plane at right angles to the sagittal plane of the seedling. This is augmented at a lower level by metaxylem, and ultimately forms what to all appearances is a normal exarch hypocotyledonary xylem group. In some seedlings the new protoxylems arise at a much lower level, and may be evident only at or near the level at which the whorl of lateral roots is produced. Exceptionally they may not be represented in the hypocotyl at all (Figs. 36, 37, 41, 42), and then are evident only as new root-poles. The fact that a series of stages is present leading from one extreme to the other leaves little doubt, however, as to their being homologous.

The production of a single additional root-pole by this method is the one most commonly occurring in typical syncotylous ('Type 1') seedlings, whilst the development of two such poles, one on either flank of the cotyledonary midrib, is very common in 'Type 2' seedlings, tetrarchy resulting in both cases. The asymmetrical development of one of these accessory strands alone in 'Type 2' seedlings is rare and results in a triarch condition, but it is interesting to note that tetrarchy may be produced by a combination of this type with an independent lateral on the opposite side and is indeed fairly common (Figs. 30-34). Only a single 'Type 1' seedling (Figs. 35-38) has been observed in which this combination of the two methods of additional xylem pole formation has occurred. Of the two poles, the one derived from the independent and persistent lateral alone gives rise to a lateral root (Fig. 37*c*), whilst the second pole (Fig. 38*e*) is only differentiated in the tap-root. There are thus four lateral roots in the root-whorl, whilst the tap-root is pentarch. The production of two new poles in 'Type 1' seedlings is extremely rare and has only been met with in two instances. One of these (Seedling A), in which the syncotyl is extremely intimate, has already been described. The second (Fig. 5) is one in which the additional poles only arise in the tap-root, which is thus pentarch, although the root-whorl consists of three members only (Figs. 39-



FIGS. 30-42. Figs. 30-34 show the transition in a 'Type 2' seedling in which tetraarchy has resulted from the production of one new pole and the independence of one lateral. The new pole does not give rise to a lateral root at the base of the hypocotyl. Figs. 35-38 show a 'Type 1' seedling with similar transition features, *a*, *b*, *c*, *d* poles, represented in the hypocotyl, *e* pole present from the base of the tap-root onwards. Note the single axillary bud. Figs. 39-42 show the transition in a 'Type 1' seedling, in which the three hypocotyledonary poles, *a*, *b*, *c*, are reinforced in the tap-root by two additional ones, *d*, *e*.

41). It will be noted that in no instance does the root-whorl consist of five members, although one 'Type 1' specimen was obtained which, on macroscopic evidence, was thought to provide evidence of such a condition. In this seedling the hypocotyl is symmetrically triarch (Fig. 44), and a lateral root arises from each of the three poles (Fig. 45, *a*, *b*, *c*). Two additional roots (Fig. 45, *adv*, *adv*¹) arise at the same level which are not referable to any protoxylem, and sections at a lower level show that the main root has been broken off and is replaced by a healed stump, on either side of which are two heptarch adventitious roots (Fig. 46). The two lateral roots arising at the level of the root-whorl are evidently adventitious also, and conform to the description given by Miss McClatchie (23), who has shown that the production of adventitious roots of this type at or near the surface



FIGS. 43-46. A 'Type 1' seedling in which the tap-root has been destroyed. Note the adventitious roots, *adv*, *adv*¹, at the base of the hypocotyl, and the two heptarch ones arising on either side of the healed stump at a lower level.

of the soil is a characteristic traumatic response in *Impatiens Roylei*. The results with regard to the derivation of the root-poles may be summarized as follows:

	'Type 1' Seedlings.	'Type 2' Seedlings.
(a) One additional pole arising <i>de novo</i> in the hypocotyl or tap-root	Common	Somewhat rare
(b) One additional pole arising by the persistence of an independent lateral	Fairly common	Fairly common
(c) Two additional poles by the combination of (a) and (b)	Very rare	Common
(d) Two additional poles arising <i>de novo</i> in the hypocotyl or tap-root	Very rare	Common
(e) Two additional poles arising by the persistence of <i>both</i> independent laterals	Unknown	Very rare
(f) One new pole arising by the bifurcation of the protoxylem	Very rare	Unknown

It is obvious that a series of modifications so suggestively similar in the two types of seedling points to community of origin, especially when, as is the case here, extremely intimate syncytely leads to the type of modification found commonly in 'Type 2' seedlings. We can thus say that points of

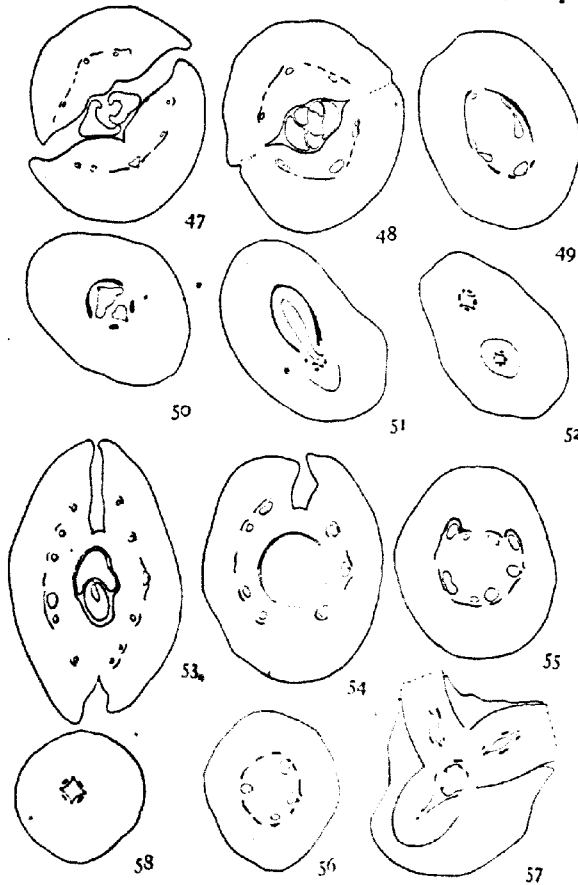
contact between the two types of seedling have been established in four respects, namely:

1. The proof that the coalescence of midribs is possible.
2. The proof that a coalescence of cotyledonary axillary buds is possible.
3. The fact that hypocotyl and root show similar types of vascular modification.
4. The fact that the modifications in the epicotyl are identical.

Quite apart from their significance as links between the two types of seedling, the vascular modifications have an additional interest as illustrating how such seedlings, whilst retaining certain ancestral features, may modify these and add to them. Syncotylous has inevitably brought with it a suppression of certain of the vascular strands, and it would seem that this modification of the normal symmetry has evoked a response in many cases consisting of either the modification in relative importance of the persistent lateral vascular components or the introduction of entirely new strands into the hypocotyl and root. It seems impossible to assign any phyletic significance to these modifications, and one is compelled to assume that the impetus to change is physiological. If we make that assumption we are confronted with the difficulty that seedlings below the average in size may show a new complexity far in advance of that shown by seedlings above the average size which have retained the simplified vascular features imposed upon them as a result of compression. It is possible that accurate measurements of the total bulk of the vascular system and of the transpiring and assimilatory areas would show that there is a definite relationship between these, but the whole problem only serves to stress our profound ignorance of many of the fundamental features of seedling physiology.

The literature of seedling anatomy contains a number of references to an increase in the number of root-poles from above downwards, and in some of these a physiological explanation of the phenomenon is suggested. Thus Sargent (26), speaking of the development of new poles in *Fritillaria alpina* and *F. imperialis*, regards this as 'due to the persistence of the primary root and its physiological activity, which renders a considerable girth necessary'; whilst Hill and de Fraine (16), referring to a similar phenomenon in the Cycadaceae, believe that the probable cause is 'that the swollen axis requires a greater dispersal of the vascular tissues'. There is, however, no obvious reason to account for the increase in the number of root-poles in cases such as that of *Opuntia Tuna*, Series B, described by de Fraine (10). While the general trend of the present investigation has been to show that the two types of seedling have a syncotylous origin, a careful search has been made for seedlings showing marked inequality of the cotyledons, and of these two have been obtained, one of which is syncotylous in addition. Neither shows any affinity with the seedlings

previously described, but both exhibit features of some interest and will be described in detail. The first seedling (Figs. 6 *a*, 6 *b*) exhibits no striking anatomical peculiarity in either the cotyledons or petioles, except that the



FIGS. 47-58. Figs. 47-52 show the transition phenomena in a seedling (Figs. 6 *a*, 6 *b*) in which the cotyledons are markedly unequal. Figs. 53-58 show the transition in a seedling (Fig. 7) in which cotyledonary inequality and syncotily are combined.

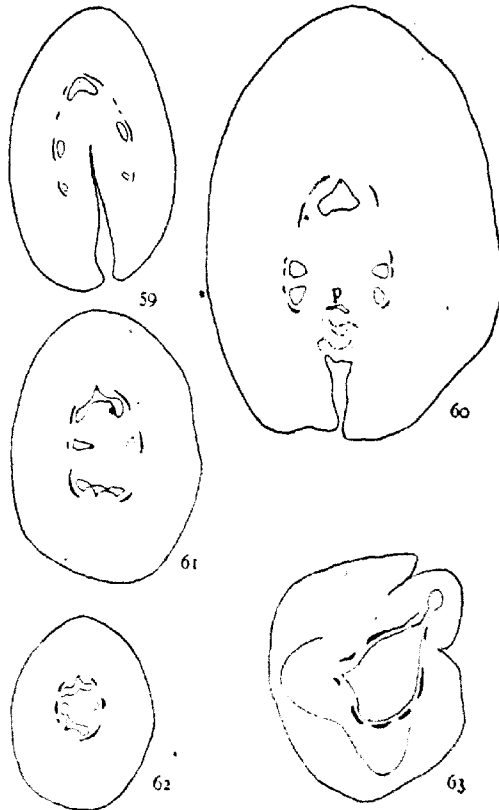
vascular bundles are smaller and more feebly differentiated in the reduced member (Figs. 47, 48). This difference in size is faithfully reflected in the hypocotyl (Fig. 49), though the transition is normal until the base of the hypocotyl is approached. In this region the intercotyledonary xylems appear to lose their protoxylem and unite with the xylem from the midrib

of the smaller cotyledon to form a large mass (Fig. 50). Only a single root is produced at the point normally occupied by the root-whorl, and this is continuous with the pole which forms the midrib of the larger cotyledon (Figs. 51, 52). The tap-root is tetrarch. It is obvious that the reduction in the size of the cotyledon exercises an appreciable effect on the vascular symmetry even at the base of the hypocotyl, and the seedling suggests comparison with *Abronia umbellata* and *A. villosa*, in which a difference in the size of the cotyledons is a normal feature. Hill and de Fraine (17), who have investigated the seedling anatomy of these two species, show that here also the vascular strands of the reduced cotyledon play a smaller part in the transition phenomena than those of the larger one, though the details of transition differ widely from those of *Impatiens Roylei*. The second specimen is remarkable in that it combines inequality in the size of the cotyledons with syncotyl, this being unilateral as regards the laminae but involving both petiole margins (Fig. 7). The bundle system of the smaller cotyledon consists of a series of collateral strands, one of which occupies the position of the midrib (Fig. 53). At the apex of the hypocotyl these bundles have been reduced to two laterally situated pairs, a phloem group marking the site occupied by the 'double' bundle in the normal cotyledon (Fig. 54). Of these, one pair, after the union of its constituent bundles, fuses with the adjacent lateral from the larger cotyledon, the vascular system of which is normal (Fig. 55). The strands of the other pair, after union, form an endarch xylem group which remains independent of the adjacent lateral and forms a pole. The lateral thus left continues down the hypocotyl for some distance (Fig. 56), but ultimately dies out, so that the hypocotyl develops a triarch symmetry and produces three lateral roots at its base (Fig. 57). Subsequently a fourth pole is developed by the tap-root in the plane corresponding to that passing through the independent lateral to which reference has just been made. The behaviour of the strands in the second seedling recalls that described for a somewhat similar case by one of us (18), in which a seedling with unequal cotyledons developed an asymmetrical triarchy in the hypocotyl owing to the absence of a 'double' bundle midrib in a smaller cotyledon. It is difficult to say whether the replacing of the normal median symmetry by a lateral bundle concentration is to be correlated with the reduction of the cotyledon or not, but this is doubtful. The available material is quite inadequate for the determination of the homologies of the bundles involved, but it is worthy of note that Davey (9), in her description of the seedling anatomy of certain Amentiferae (e.g. *Juglans cinerea*, *Pterocarya rhoifolia*), records a loss in the relative importance of the median cotyledonary strand and a corresponding increase in the transition value of the laterals, though the similarity between these forms and *Impatiens* is not a close one.

The formation of a very short cotyledonary tube at the base of the

petioles is a common feature of normal seedlings and occurs in many of the abnormal seedlings also (Fig. 43). Occasionally, as in the seedling just described, the whole of the petiole is involved and leads to an asymmetrical amphisyncotyly. This condition may exceptionally cause a partial roofing-in of the plumular bud owing to secondary fusions of the apposed cotyledonary tissues. The effect of this roofing-in is apparently in many cases to cause a partial arrest of plumular development, since, as a general rule, the plumule is ill developed compared with that of other seedlings of similar size and age in which the cotyledonary tube is rudimentary or absent. The seedling illustrated in Fig. 8 is a case in point. Both from the standpoint of size and the amount of secondary thickening present this seedling should have possessed a partially expanded epicotyl, but as a matter of fact it shows a stage of development very little in advance of that possessed by quite young seedlings. Its position is indicated by a large swelling in the basal portion of the tubular petiole, and some idea of the relatively enormous size of this region may be gleaned from a comparison of Fig. 60 with that of the same region in other seedlings. The cotyledonary tube communicates with the exterior only by a small pore (Fig. 60, *p.*), and the adjoining tissues show evidences of compression in the rows of flattened cells which are indicated by dotted lines in the diagram. The vascular details of this seedling also serve to illustrate one or two points of interest. The midrib forms one xylem pole in the hypocotyl and the marginals constitute another, both laterals remaining independent and showing an endarch position of the protoxylem (Fig. 61). One of the independent laterals persists throughout the hypocotyl but loses its protoxylem, whilst the other soon dies out completely, its position being represented for a short distance by a small patch of secondary xylem (Fig. 61). Towards the base of the hypocotyl a new bundle appears in the same relative position (Fig. 62), and though it does not give rise to a root at the root-whorl, which consists of three members, it is continued downwards and forms a root-pole. It will be noticed that the history of this particular root-pole is capable of two interpretations, since it may be derivable either from the independent lateral, which died out after a brief course in the upper part of the hypocotyl and has subsequently been restored, or it may be an entirely new structure. The second view seems the more probable, since in all other cases observed, with the exception of 'Seedling A', and of the syncotyl with unequal cotyledons previously described, where an independent lateral dies out in the upper hypocotyl, no root-pole of any kind is formed in the corresponding position. Hill and de Fraine (16) record a case in *Pinus sylvestris* in which there is a local disappearance of a vascular strand, and similar cases have been noted by other investigators; but these are hardly comparable with the case under discussion, in which a distance of over three centimetres separates the termination of the one strand from the initiation of the other. Amphisyncotyly involving both

laminae was not found so commonly, but where it does occur the fused blades have either a salver-like appearance (Figs. 9, 10 *a*, 10 *b*, 11), in which case the upper portions of the laminae are free, or are goblet-shaped with the whole length of the blade margins united (Figs. 13 *a*, 13 *b*, 13 *c*). The effect



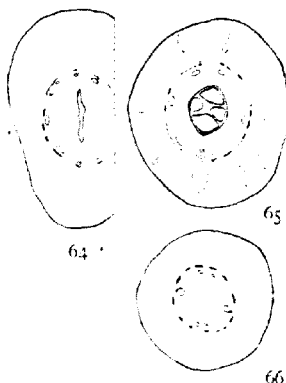
FIGS. 59-63. Transition in a 'Type 2' seedling (Fig. 8). Fig. 60 shows the effect of partial roofing-in of the plumule on the side of the base of the petiole. The dotted lines indicate irregular lines of crushed cells; p. = the pore-like communication with the plumular bud.

on the anatomy is exceedingly variable, but in nearly every case a more or less complete roofing-in of the plumular bud is evident (Fig. 64). The two-sided fusion of the cotyledons evidently imposes considerable strain on the tissues, as in some cases a tearing has occurred down one side (Fig. 14) whilst in others lacunae are developed in the mesophyll, which can only be

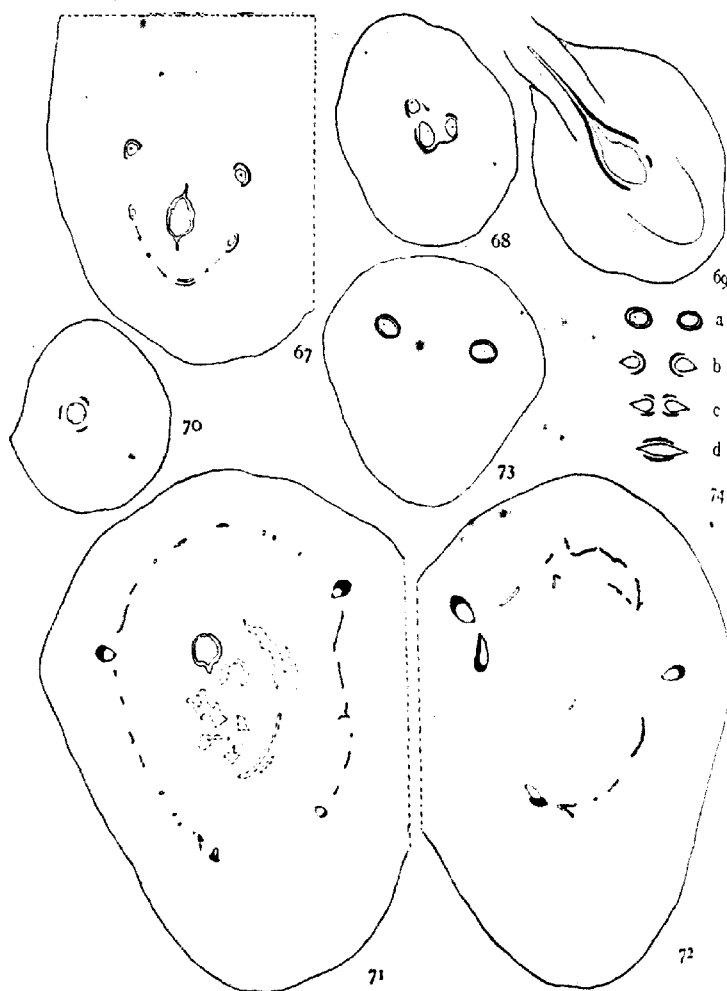
ascribed to the conflicting internal tensions set up (Fig. 65). In some cases the transition features show no noteworthy variation from those of normal dicotyls (Fig. 66), but several show rather remarkable modifications, and some of the most clearly defined of these are described below.

'Seedling C' (Fig. 9) is an amphisyncotyl showing a salver-shaped condition. The marginals and laterals of both cotyledons on one flank unite with their respective midribs at a fairly high level, thus causing the latter to appear to have undergone displacement to one side (Fig. 67). The midribs themselves are mesarch and are almost enclosed by their phloems, which form strongly incurved arcs (Fig. 67). The bundle systems of the other side gradually unite until they form a large mesarch xylem mass, the phloems of which are linked locally with the adjacent half-phloems of the midribs (Fig. 68). This xylem mass loses its protoxylem and gradually becomes very much reduced, being represented at the base of the hypocotyl by a little metaxylem only. The midribs alone contribute to the root-whorl, which thus consists of two members (Figs. 9, 69), and below these a protoxylem is developed in the plane of the third hypocotyledonary xylem group, the tap-root being triarch.

'Seedling D' (Figs. 10 a, 10 b) is externally a seedling of similar type to the previous one, but its vascular arrangements are somewhat different. The midribs are well defined, as are the marginals of one side, but generally speaking the lateral systems of both cotyledons consist of an elaborate anastomosing series of small veins which may locally extend into the solid basal tissue formed by the secondary ingrowths from the adaxial petiolar surfaces. The well-defined marginals after a temporary union diverge, and the whole of the bundle complex gradually concentrates upon the two midribs, uniting with these adaxially so that a pair of mesarch xylem groups, completely surrounded by phloem, enters the hypocotyl (Fig. 73). Traversing the hypocotyl the xylem becomes exarch and the sheaths of phloem open at the protoxylem (Fig. 74, b), so that for a time they are U-shaped in transverse section. A little lower down they divide into two parts which unite laterally, the xylems becoming united at the same level, so that a diarch plate results (Fig. 74, c, d). Two lateral roots are given off at the base of the hypocotyl, and the tap-root is diarch.



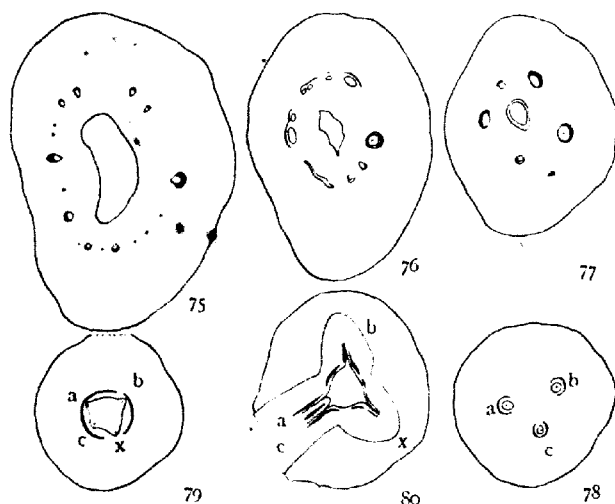
FIGS. 64-66. An amphisyncotyl (Fig. 11) in which partial roofing-in of the plumule has occurred, and in which the cortex shows splits due to tissue tensions.



FIGS. 67-74. Figs. 67-70 illustrate the transition phenomena in 'Seedling C' and Figs. 71-74 those in 'Seedling D'.

'Seedling E' (Fig. 12) is a goblet-shaped amphisyncotyl. The vascular system shows no apparent abnormality in the upper part of the fused laminae, but at the base neither midrib develops 'double' structure. A concentration of the majority of the lateral strands upon one collateral

midrib occurs, leading to the formation of a pseudo-concentric bundle. The other also becomes pseudo-concentric. A minor concentration also takes place on one of the laterals (Fig. 78, *b*), so that three mesarch bundles pass into the apex of the hypocotyl. Each of these is for a time completely invested by phloem, but as the bundles move towards the axis of the seedling the phloems open on the inner side and their free margins unite so that a common ring surrounding the whole of the xylem is formed. The continuity of this ring is permanently broken opposite the now exarch protoxylems and also locally throughout, but otherwise there is no further change until the formation of the solid xylem core, which heralds the formation of

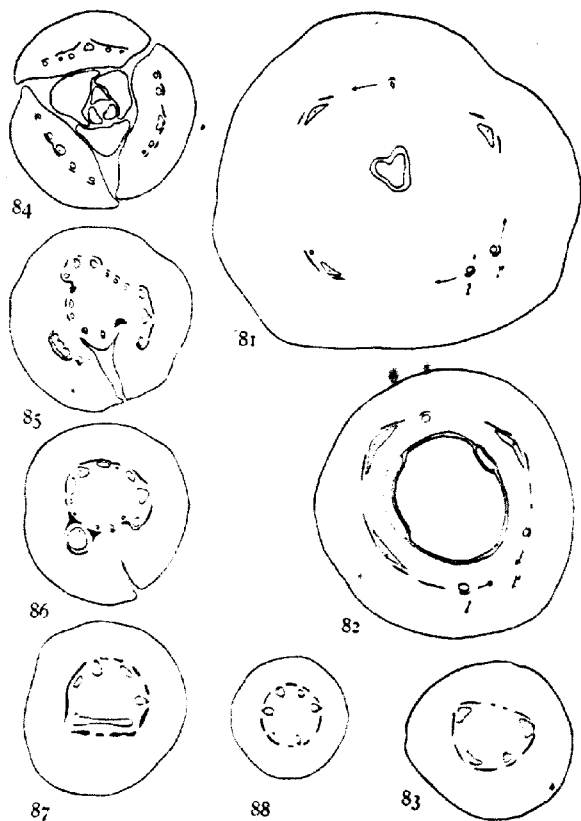


FIGS. 75-80. A goblet-shaped amphisyncotyl, showing an abnormal type of transition.

the lateral roots, is well forward. At this stage a new protoxylem pole arises between the strand continuous with the permanent lateral and one of the midrib strands. This pole gives rise to a lateral root, as does one of the midribs (Fig. 80), and the third lateral root of the series appears to be derived from the lateral and the second midrib jointly, a most unusual state of affairs. The tap-root is tetrarch, the four poles represented in the base of the hypocotyl persisting unchanged.

'Seedling F' (Figs. 13 *a*, 13 *b*, 13 *c*) is also goblet shaped, and sections reveal the fact that it possesses three fused cotyledons, that is to say, it is an amphitrisyncotyl. A trisyncotylous seedling of *Centranthus ruber* has been described by Miss Bexon (1), and a somewhat similar seedling of *Acer pseudoplatanus* has been collected by one of us, but neither of these exhibits

the symmetrical amphisyncotyl of the subject of the present observations. A section towards the base of the cotyledons shows three somewhat flattened mesarch double bundles with which the laterals have either united or are converging to do so (Fig. 81). At a slightly lower level the flattening of the double bundles is more pronounced, and the cotyledonary axillary buds are



FIGS. 81-88. Figs. 81-83 illustrate the earlier stages of transition in an amphitrisyncotyl (Figs. 13 a, 13 b, 13 c); Figs. 84-88, the earlier stages of transition in a tricotyl.

evident, two of the three showing partial union to form a tangentially elongated structure (Fig. 82). One pair of laterals (Figs. 81, 82, *l, l'*), after uniting with their respective midribs, separates again and unites in the intercotyledonary plane to form a collateral endarch group, which, however, dies out in the hypocotyl. The three main bundles, which at this level are

exarch, are continued downwards without any striking modification, and a triarch condition characterizes the remainder of the seedling. The evidence provided by the study of a relatively small number of amphisyncotyls hardly justifies the putting forward of any decided views as to the effects of this type of cotyledonary fusion, but there does seem to be a definite tendency towards the modification of the lateral strands, this taking the form of relatively early union of these with the midrib instead of their pursuing an entirely independent course.

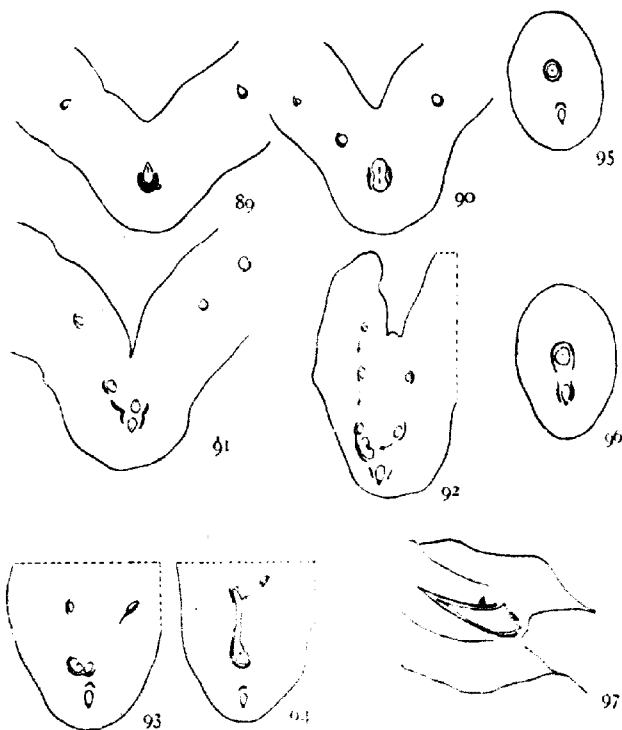
Two other seedlings remain to be described which show special anatomical peculiarities and which constitute at present more or less isolated records. The first of these, 'Seedling G', is a tricotyl, and exhibits trimerous symmetry in the epicotyl (Fig. 84). The general vascular features of each cotyledon are apparently quite normal, but the midrib of one retains its collateral structure to the base of the petiole when that of the other two is distinctly 'double' (Fig. 84). As the node is approached the petioles fuse, but the adjacent margins of two show delay compared with the remainder (Fig. 85). At this level the vascular strands of that cotyledon possessing a collateral midrib coalesce to form a tangentially elongated group (Fig. 85), which later loses all trace of its triple origin and forms an endarch xylem group almost surrounded by phloem (Fig. 86). This type of structure has been regarded as an 'Anemarrhena' condition by Lee (21), Thomas (31), and others, but it is a far cry from a temporary union such as we have here, even where it leads to local loss of identity, to the condition recorded by Sargent (24) for *Anemarrhena*. The subsequent history of the aberrant vascular structure is peculiar. It will be perceived that the transition in the other two cotyledons is normal, the adjacent laterals of these members uniting to form a xylem group in the intercotyledonary plane, and each leaving a free lateral on the side adjoining the remaining cotyledon. Near the apex of the hypocotyl the bulk of the large strand turns almost horizontally to unite with one of the free laterals (Fig. 87), whilst at a slightly lower level a smaller portion unites with the free lateral of the other cotyledon, a small remnant thus being left. A section at this level would therefore show the following strands:

1. A pair of exarch strands derived from the midribs of two of the cotyledons (Fig. 88).
2. A pair of exarch strands derived from the laterals and parts of the vascular system of the aberrant cotyledon (Fig. 88).
3. A strand produced by the union of the adjacent laterals (Fig. 88).
4. A small strand representing the remnant of the vascular system of the aberrant cotyledon.

Of these, the strand produced by the union of the laterals remains endarch and dies out in the upper part of the hypocotyl, the small strand, which consists of metaxylem only, sharing the same fate. The four exarch

trands thus left continue throughout the remainder of the seedling, producing a root-whorl of four members and a tetrarch tap-root.

'Seedling H' is a 'Type 2' seedling in which the anatomy of the major portion of the blade shows no striking difference from its fellows (Fig. 89). Towards the base of the lamina, however, the midrib becomes hour-glass shaped in transverse section (Fig. 90), and the protoxylem, which is mesarch at this stage, elongates and separates into two, half supplying the adaxial



FIGS. 89-97. A 'Type 1' seedling, showing an abnormal type of transition.

portion of the bundle and the other half supplying the outer portion. The adaxial portion then separates off as a separate mesarch strand (Fig. 91) which soon becomes endarch, whilst the outer portion becomes exarch and forms a typical 'double' bundle. The endarch portion then assumes a slightly lateral position and is joined successively, first by two lateral strands (Fig. 92) from the side to which it is inclined, and then by one from the opposite side (Fig. 93). Finally the marginals from both flanks (Fig. 94) unite and together join the xylem complex previously formed, the

whole forming a mesarch xylem completely surrounded by phloem (Fig. 95). Meanwhile the phloem of the double bundle has bent inward and the two halves have united adaxially (Fig. 95). The two strands thus formed traverse the greater part of the hypocotyl unchanged, but near the base the phloems open out on the inner side and unite laterally, the mesarch bundle at the same time becoming exarch and causing a break in the phloem at this point also (Fig. 96). Two lateral roots are developed and a new protoxylem originates in the plane at right angles to these (Fig. 97), so that the tap-root is triarch. The interpretation of the singular vascular behaviour of this seedling is distinctly difficult, though it shows points of considerable resemblance, especially in the concentration towards the middle line of the lateral strands, to a similarly odd seedling previously described by one of us (18). The division of the midrib as the petiole is approached suggests that not only have the two phyletically distinct midribs merged their identity, but that one of the lateral strands has also become involved in the intimate union. In the absence of further supporting evidence, however, such an explanation must be regarded as purely tentative, and the chief interest of the seedling is that it provides a further instance of the plastic nature of juvenile vascular structures.

DISCUSSION.

The investigation of the structure of seedlings, such as those described in the present paper in which a pseudo-monocotyledonous condition is a relatively common teratological feature, provides a tempting starting-point for a consideration of the possible origins of the Monocotyledons proper. Theoretically the seedling possessing a single leaf is derivable from the dicotyledonous type in a variety of ways. These may be briefly tabulated as follows:

1. By syncotyly involving both cotyledonary margins (amphisyncotyly) so that a cotyledonary tube, often more or less solid secondarily, results. This condition is occasionally realized in *Impatiens Roylei*.
2. By syncotyly involving one margin only of the originally separate cotyledons. This is the condition most frequently found in the syncotyls of *I. Roylei*.
3. By a specialization in function of the cotyledons, one of which becomes purely suctorial, the other undergoing retardation in development and subsequently appearing as the first leaf.
4. By a total suppression of one cotyledon.
5. By the suppression during embryogeny of the meristematic activity of the cotyledonary apex, the other cotyledon, with the basal sheath common to both, continuing to develop and producing the typical sheathing structure which characterizes the majority of Monocotyledons.

Of these the first alternative has received strong support from Sargent (26) as the result of an extensive series of comparative observations on the seedling anatomy of the Monocotyledons and of certain Dicotyledons, in which an amphisyncotylous condition obtains. This worker considers that the type of seedling anatomy characterizing *Anemarrhena asphodeloides* provides the clue to the source of the monocotyledonous condition. In this species the major portion of the cotyledon is traversed by two separate symmetrically placed strands, each of which, at the level of transition to the primary root, branches into three, the marginal portions uniting in pairs and constituting two of the root-poles, whilst the median portions also each form a root-pole, so that tetrarchy results. The 'Anemarrhena' condition is believed to have given rise in other Monocotyledons to that type of cotyledonary vascular supply in which there is a single median cotyledonary bundle (e. g. *Allium*, *Zygadenus*) by the intimate fusion of the two originally independent strands. The 'Anemarrhena' condition is further compared with the type of vascular symmetry shown by a dicotyledonous species, *Eranthis hiemalis*, in which the seedling is amphisyncotylous. In this species each cotyledon is supplied with a median bundle flanked by lateral strands, the latter uniting with the midrib at the base of the lamina. Each midrib bifurcates in the hypocotyl so that two pairs of independent collateral bundles result. From these what is interpreted by Sargent as a fugitive tetrarch condition ultimately results, diarchy following on the loss of the two intercotyledonary protoxylems. The development of an Anemarrhena-like phase in the hypocotyl and root of a geophilous dicotyledon is considered to support the view that Monocotyledons have arisen by a method approximating to this. De Fraine (10) criticizes this interpretation and points out that the occurrence of an Anemarrhena-like phase is not confined to geophilous Dicotyledons. (It has been recorded, for example, by Thomas (31) in *Althaea*, by Lee (21) in *Incarvillea*, and by de Fraine (10) in *Opuntia* spp.) It is noted also that amphisyncotily does not necessarily lead to the type of anatomy characterizing *Anemarrhena* (e. g. *Delphinium* sp., *Anemone coronaria*). A further criticism is put forward by Thomas (31), who considers that the xylem elements regarded by Sargent as constituting the intercotyledonary poles in *Eranthis hiemalis* are probably of secondary character, and if this view is correct the species is diarch throughout and the resemblance to *Anemarrhena* is illusory. Much obviously depends upon the correct interpretation of the two independent strands which traverse the cotyledon in *Anemarrhena*. If each represents the products of an intimate fusion of a median strand with a lateral on either flank, then the resemblance to Dicotyledons of the *Althaea* type is plain. There is, however, a possible alternative, namely, that each represents the widely separated half of a 'double' bundle which has fused with a lateral and a marginal strand on the distal flank only. That a condition analogous to this is possible is

shown by Chauveaud (4) for *Cordyline indivisa*. Moreover, the same author has also shown that typically the lower portion of the cotyledonary midrib, or the hypocotyledonary strand derived from it, is initiated as a radial file of xylem elements flanked by the phloem groups, this arrangement giving place with advancing age to two separate collateral strands, that is, to an *Anemarrhena*-like condition. Further, a number of cases are on record in which the earlier phases (Chauveaud's 'Alterne' and 'Intermédiaire' groupings) do not occur in ontogeny, this being notably the case in *Podocarpus chinensis* and *Ephedra distachya* (16), in which the half-bundles are separate throughout the cotyledon and hypocotyl. Sporadic cases also occur in which one of the cotyledonary midribs is represented by a 'double' bundle, while the other is represented by two wholly independent half-bundles (e.g. *Echinocereus Ehrenbergii* (10)). We have no information as to the very early vascular phases of the majority of the seedlings with which Sargent worked, but it seems plain that the widely separated strands characterizing *Anemarrhena* may be as readily regarded as the ultimate derivatives of a series starting from a compact 'double' bundle (that is, one consisting of a central xylem group flanked by phloems) as the initial member of a series which has evolved the compact 'double' bundle as a result of fusion. Which of these alternatives is the correct one cannot be stated with confidence, but the probabilities appear to lie with the former. The special type produced by the merging of the identity of the lateral strands in that of the main bundles does not appear to have the phyletic significance which Sargent would attach to it, but it serves to illustrate the fact that members of widely different groups (e.g. *Anemarrhena*, *Opuntia*, *Althaea*, *Incarvillea*) may show a common type of vascular evolution. De Fraine (10) further, as a result of her studies of the seedling anatomy in the Cactaceae, also casts doubt on the phyletic significance of the 'double' bundle, pointing out that this structure, in the hypocotyl, may be the product of widely different contributing strands. It must be borne in mind, however, that it is only in the more specialized cactaceous seedlings, forms in which the cotyledons are vestigial and in which the hypocotyl is short and much thickened, that the hypocotyledonary strands are produced in this exceptional manner, and it is very doubtful whether evidence derived from the study of such forms should be regarded as invalidating a view which is supported by the large series of facts derived from the investigation of more normal seedlings.

However, while it is perhaps justifiable to say that the grounds upon which Sargent has based her conception of the *amphisyncotylous* origin of the Monocotyledons are open to somewhat damaging criticism, there is a certain amount of evidence which seems to support the view that a *one-sided syncotylous* may have given rise to a monocotylous condition. Sargent indeed accepts this view of the origin of the pseudo-monocotyledonous

Dicotyledons, and Compton (6), whilst apparently favouring the derivation of the *Anemarrhena* type through amphisyncotyly, considers that the exalbuminous Helobieae may have arisen by unilateral syncotyly. It is noteworthy that in *Impatiens Roylei*, which possesses an exalbuminous seed with a perfectly straight embryo, both amphisyncotyly and unilateral syncotyly occur, the latter predominating. The evidence as to the origin of the 'Type 2' seedlings of *Impatiens Roylei* brought forward in the present paper points strongly to their being derived from a dicotyledonous type by a close unilateral syncotyly, yet a study of a typical member of this group shows no sign whatever of the dual origin of the cotyledonary structure. If this evidence is accepted, then we are bound to admit that a midrib phyletically the product of two 'double' bundles behaves throughout in a manner identical with that of one of the ancestral midribs. The evidence put forward by Thomas and Davey (32) in the cases of *Anemone apennina*, *Ranunculus Ficaria*, and *Conopodium denudatum*, as far as can be judged from the published abstract, also seems to point in the same direction. A further point in favour of unilateral syncotyly, and one again the significance of which Davey and Thomas seem to recognize, lies in the position of the first epicotyledonary leaf relative to the median plane of the cotyledons. It is extremely rare to find the first epicotyledonary leaf, in Dicotyledons, lying in any other plane than that at right angles to the median cotyledonary plane. The position of the first leaf in Monocotyledons, granted unilateral syncotyly, is strictly conformable with that typical with the vast majority of Dicotyledons. On the other hand, unless it is assumed that in the Monocotyledons there has been a shifting through 90° of the position of this leaf, it must remain a stumbling-block to all theories in which differentiation of cotylar function or partial or total suppression of the second cotyledon is considered as the source of Monocotyledony. The support of the theory of the differentiation of cotyledonary function is largely due to A. W. Hill (14), and has resulted from his investigation of the seedling anatomy of a number of geophilous species of *Peperomia* which yield a series of forms commencing with a normal dicotylous type and ending in one in which one cotyledon is purely suctorial in function whilst the other is purely assimilatory. Hill considers that monocotyledony has arisen as a result of specialization of this kind, the assimilatory cotyledon having delayed its development until carried outside the seed, when it appears as the first leaf. Hill regards the monocotyledonous seedling of the Araceae as conforming more nearly to the primitive condition, and would group Sargent's *Anemarrhena-Zygadenus* series in the reverse order to that adopted by her. De Fraine (10) supports this view, considering that the continued separation of the components of the 'double' bundle into two independent portions would, on physiological grounds, be advantageous in an absorbing cotyledon and would thus lead to the condition charac-

terizing forms like *Anemarrhena*. There is no evidence, however, of any connexion between the absorptive cotyledonary function and the separation of the halves of the 'double' bundle, and in the absence of such evidence this idea can only be regarded as purely speculative. It has been pointed out already that the orientation of the epicotyledonary leaves presents a difficulty against which this theory has to contend, and it is noteworthy that in *Impatiens Roylei* undoubtedly syncotylous seedlings exhibit that balance of cotyledonary structures and first epicotyledonary leaf which Hill has laid stress upon in certain Aroids (e.g. *Arisaema dracontium*). Apart from this, however, there seems to be no reason to consider Hill's view as unlikely, nor does its acceptance automatically eliminate the possibility of a syncotylous origin for some groups of Monocotyledons. Certain systematists, notably Lotsy, would regard the Monocotyledons as at least diphyletic in origin, deriving the Spadiciflorae from an ancestral dicotyledonous group approximating to the Piperales, and the remaining forms from a hypothetical pro-ranalean stock which has also given rise to the modern Ranales. The derivation of the Monocotyledons from the Dicotyledons by the complete suppression of one cotyledon depends for support largely on indirect evidence. Many undoubted Dicotyledons are known in which there occur seedlings showing a marked dissimilarity in size between the two cotyledons (e.g. *Abronia* spp., *Citrus Aurantium*, *Pachira aquatica*). Occasionally a similar phenomenon occurs in *Impatiens Roylei*, and the anatomy of three such seedlings has already been described, two in the present and one in the previous paper (18). Other Dicotyledons, again, are known in which only a single cotyledonary member is produced normally (e.g. *Cyclamen persicum*, *Ranunculus Ficaria*, *Carum bulbocastanum*, *Corydalis tuberosa*). Of the first group *Abronia umbellata*, *A. villosa* (17), and *Impatiens Roylei* are, as far as can be ascertained, the only species in which the anatomy has been investigated. These undoubtedly show that where a pronounced inequality in the size of the cotyledons exists this exercises a marked effect on the vascular symmetry of the hypocotyl and, in a measure, of the root, the strands contributed from the smaller cotyledon being less important than those of the larger one. With regard to the pseudo-monocotyledonous types the available evidence, though as yet somewhat meagre, points to their being syncotylous in origin in many cases (32). The all-important cases which link the species with unequal cotyledons with those which are apparently pseudo-monocotyledonous are thus lacking, and in their absence the 'suppression' hypothesis must be regarded as not proven.

The most recent theory as to the origin of the Monocotyledons is that put forward by Coulter and Land (8), who consider that there exists both in the Monocotyledons and Dicotyledons a peripheral cotyledonary zone which gives rise to two or more growing points or primordia, this being followed by a general zonal development and resulting in a cotyledonary ring or

sheath of varying length. If both growing points continue to develop equally the dicotyledonous condition is attained. If one of the growing points ceases to develop, the growth of the whole cotyledonary zone is associated with that of the other growing point and the monocotyledonous condition is reached. Monocotyledony is thus due neither to syncotily nor to cotylar suppression, but is simply the result of the continued development of one growing point of the cotyledonary ring rather than a division of the growth between two growing points. Cotyledons are thus invariably lateral structures. The variation in the cotyledon number which has led to the separation of the two great Angiosperm series is considered to have arisen, in all probability, in those forms which possessed a massive proembryo of a type similar to that of the Liliales, Arales, and many Ranales among the modern groups. The massive proembryo is therefore held to represent the primitive proembryonic condition, the filamentous type of *Alisma* and *Capsella* being derived from this.

The evidence marshalled in support of this theory lies chiefly in the interpretation placed upon the intermediate stages of embryonic development in certain Monocotyledons, notably *Agapanthus umbellatus* and *Cyrtanthus sanguineus*, but also on the comparative morphology of monocotyledonous and dicotyledonous seedlings of the former. The fact that *Agapanthus* produces, though apparently rarely, dicotyls as well as monocotyls is considered to be significant, since, it is argued, if this is the case there must be some evident relationship between the two conditions.

The vascular system of the normal seedlings of *Agapanthus* is simple. The cotyledonary vascular supply consists of a median bundle with a lateral on either flank. These unite at the node, where they are joined by the midrib of the first plumular leaf. The strand from the latter forms a root-pole, whilst the cotyledonary strands between them form two poles so that a triarch root is organized. At a lower level a tetrarch condition arises owing to the bifurcation of the pole derived from the first leaf-strand. From the fact that the primordia of the cotyledon and first plumular leaf are developed first and the vascular strands subsequently laid down in these, it is inferred that the vascular features are determined by the primordia rather than the reverse. The vascular strands, in fact, are held to be 'secondary structures whose appearance is dependent upon the character of the primary structures and therefore of no phylogenetic significance'.

The dicotylous specimen described shows two epicotyledonary leaves also. Each cotyledon is supplied with two strands in lateral positions 'as if the middle one present in the monocotyledonous seedling has not been laid down'. Each epicotyledonary leaf has a single median strand. Six strands thus unite at the cotyledonary node, and from these, as in the monocotylous seedlings, three root-poles are organized, an additional pole being developed by the bifurcation of that continuous with the midrib of

the first plumular leaf. The possibility of the dicotyledonous embryo being a fusion twin is rejected on the vascular evidence which shows that 'it is merely a slight modification of the monocotyledonous one and gives no suggestion of the fusion of two embryos'.

In *Cyrtanthus sanguineus* (8, 12) the embryo, prior to maturation, passes through a phase in which the cotyledonary tube shows division into four shallow apical lobes, each of which is supplied by an independent procambial strand. The lobes are obviously in pairs, one of which is slightly smaller than the other. This stage Coulter and Land regard as representing a tetracotylous condition. It is followed by a stage in which, owing to a more active growth of the cells of the cotyledonary zone lying between the growing points of each pair, a union in twos occurs, this being accompanied by an elongation of the sheath. This stage is interpreted as a dicotyledonous condition. Subsequently one 'cotyledon' develops rapidly whilst the growth of the other ceases, so that a monocotyledonous mature embryo is produced in which the originally independent vascular strands from the shorter side bend sharply to unite with those supplying the elongated portion. In a further contribution Coulter (7) extends the theory to the Gramineae, homologizing the epiblast with the second reduced cotyledon, and the scutellum with the dominant one. A series is described ranging from species such as *Zizania aquatica* and *Oryza sativa*, in which the epiblast is well developed, through those showing a small epiblast (e.g. *Triticum vulgare*), to species like *Zea Mays*, in which it is absent. These are interpreted as illustrating the progressive stages in the abortion of the second cotyledon.

It will be noticed that Coulter and Land's theory rests upon three assumptions, namely that—

- (a) The massive proembryo is more primitive than the filamentous one.
- (b) The earlier and intermediate stages in embryogeny have a profound phyletic significance.
- (c) The phyletic value of the vascular structures is negligible.

All three assumptions are at least debatable, and the third seems open to serious objections. It is plain that the denial of the phyletic value of vascular structures in phanerogamic seedlings carries with it the onus of supplying an adequate alternative explanation for certain well-defined anatomical characters. Two examples of this kind may be cited. It is a matter of common knowledge that the hypocotyl in its young stages usually shows an exarch position of the protoxylem elements and a radial grouping of the xylem and phloem, this being subsequently replaced by a collateral grouping of the vascular tissues owing to the resorption of the first-formed elements and the substitution for them of others with a different orientation. The remarkable constancy with which this cycle is repeated in all phanerogamic groups (4) creates a strong *prima facie* case

for regarding it as of phyletic importance. That it is occasionally of very brief duration or that in a few species it may be altogether omitted is only to be expected, and in no wise affects the conclusions drawn from its general occurrence.

A further illustration of the value of vascular evidence of a somewhat different type is furnished by the varying behaviour of the cotyledonary strands in teratological polycotyls. In some such the midrib retains its collateral structure and, with a similar strand from a neighbouring cotyledon, constitutes a single root-pole. Such cotyledons are held by us to have been primarily produced by the qualitative division of the growing apex of the seed-leaf. In other cases the cotyledonary strand develops 'double' structure, and may either unite with a similar strand at varying levels in the hypocotyl or root or remain entirely separate and constitute an additional root-pole. These types are interpreted by us as having arisen by the quantitative division of the cotyledonary apex, and have led to absolutely symmetrical triarchy and tetrarchy in normally diarch species. Such an hypothesis provides a perfectly logical and consistent explanation of the origin of teratological polycotily, and appears to be equally applicable to the case of those Gymnosperms which possess polycotyledonous seedlings. If this hypothesis is rejected it remains to be explained why growing points which, according to Coulter and Land's theory, are presumably equivalent should capriciously produce strands which behave in transition in such varying ways.

Moreover, it is surely illogical to confine the argument to the special case of seed-bearing plants, and its absurdity is evident when we attempt to apply it to the vascular Cryptogams. As an example we may take the ontogenetic sequence in the Filicales, which is familiar enough to need no detailed description and the phyletic value of which is undoubted. Evidence of yet another kind is provided by the discovery of petrified plant remains from the Old Red Sandstone (of Middle or Lower Devonian age) of Aberdeenshire. The aërial parts of these plants, the anatomy and morphology of which have recently been described by Kidston and Lang (20), are, with one exception, entirely devoid of leaves and consist of dichotomizing assimilatory branch systems with apical sporangia. The existence of such types, which represent the oldest known vegetable petrifications, would seem to lend support to the contention that vascular differentiation phyletically antedated differentiation into stem and leaf.

Proceeding, however, from general criticism to an examination of the special evidence upon which Coulter and Land's theory rests, it will be seen that much depends on the interpretation placed upon the dicotyledonous seedling of *Agapanthus*. It seems to us that the weakness of Coulter and Land's interpretation lies chiefly in the facts which it does not attempt to explain. It is not clear, for example, why one of the two growing points,

which normally produces three strands, should only produce two, and the other, which normally produces none, should also produce two, and further why the bundles of the two 'cotyledons' should together produce a vascular condition essentially similar to that of the normal monocotyl. Another difficulty is encountered in the position of the first epicotyledonary leaf, which, in the dicotyl, lies in the plane at right angles to the median plane of the two 'cotyledons'.

Now where only one cotyledon elongates the median plane of the first epicotyledonary leaf *corresponds* to that of the two 'cotyledons'. If therefore the dicotyledonous condition is the result of the equal development of the two growing points which, according to Coulter and Land, are normally present in Monocotyledons, it has been accompanied by a shifting through 90° of the insertion of the first leaf, a phenomenon which seems somewhat unlikely. The alternative explanation is that the dicotyledonous seedling has arisen by the qualitative fission of a normally single cotyledon. In such a case each half-cotyledon would contain two bundles, namely one lateral and half the midrib bundle, and these would behave in transition like the bundle system of the undivided cotyledon, precisely as they do in the seedling under discussion. It is noteworthy in this connexion that the median cotyledonary bundle normally produces two widely divergent half-bundles prior to the constitution of the cotyledonary plate, so that complete division would only be an extension of such a process. Granted the accuracy of this interpretation the orientation of the first epicotyledonary leaf is seen to be perfectly normal, a view which is confirmed by the relationship of its vascular strands with those of the two cotyledonary members. With regard to the theoretical construction placed upon the embryonic structure of *Cyrtanthus sanguineus* it would appear that some knowledge of the earlier phases of embryogeny is desirable, since the shallow apical lobing of the younger of the two stages figured by Miss Farrell (12) may be interpreted with equal plausibility as the *result* of the development of four procambial strands rather than as the cause of their development. Finally, with regard to the conception of the epiblast of the grass embryo as a cotyledon, one would expect, if this were the case, to find at least some evidence of vascular tissue where this structure is well developed. Actually there is no trace of such tissue, unless the small meristematic group of cells opposite the scutellum in the maize, a form in which the epiblast is absent, be interpreted as such. Here too, as in previous cases, the position of the first epicotyledonary leaf offers a difficulty which Coulter does not attempt to explain. It seems to us, therefore, that, taken as a whole, the evidence submitted in support of this theory is of too flimsy and too equivocal a character to be entirely trustworthy, and certainly does not appear to provide an adequate foundation upon which to erect the imposing superstructure for which its authors have intended it.

It finally remains to state the conclusions to which our investigations have brought us. Briefly we consider that, whilst in the present state of knowledge dogmatism as to the origin of monocotyledony would be most unwise, the view that it is the result of unilateral syncotyl has much to recommend it. It is not claimed that it is necessarily the sole method by which the monocotyledonous condition may have been attained, but it is held that it provides a consistent explanation of the observed facts in Monocotyledons proper and that it is one the evolution of which is well illustrated by both normal and teratological Pseudo-Monocotyledons.

SUMMARY.

1. *Impatiens Roylei* produces two main types of abnormal seedling, one of which is obviously syncotylous (Type 1) and the other, judged by morphological standards, heterocotylous (Type 2).

2. The apparently heterocotylous type is in all probability the product of extremely close syncotyl.

3. This conclusion is supported by the following facts:

(a) Undoubted syncotyls occur in which the midrib bundles unite and behave in transition in a manner indistinguishable from that of a single normal midrib.

(b) Syncotyls occur in which there is a progressively closer fusion of the originally separate cotyledonary axillary buds.

(c) 'Type 2' seedlings occur in which the double origin of the bud is evident.

(d) The modifications found in the epicotyl are very similar in both types of seedling.

(e) The modification of the existing vascular components and the introduction of new vascular strands are fundamentally the same in both.

4. The production of new vascular strands or the modification of existing ones is believed to be a reaction against the elimination of certain strands on the symphysis side, and to be explicable on physiological grounds.

5. Amphisyncotylous seedlings occur, and these show in many cases a retardation in the development of the plumular bud which is held to be due to the partial roofing-in of this structure by the secondary tissue fusions occurring between the adaxial cotyledonary surfaces.

6. Many amphisyncotyls show vascular modifications which appear to be related to their morphological conditions.

7. The anatomy of a tricotyl and of an amphitrisyncotyl is also described.

8. It is considered that the evidence submitted as to the origin of the 'Type 2' seedlings of *I. Roylei*, combined with that derived from the studies

of other workers on the anatomy of teratological syncotyly and of species normally pseudo-monocotyledonous, renders probable the view that many Monocotyledons have arisen from Dicotyledons by a process of unilateral syncotyly.

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492 *Holden & Daniels.*—*Anatomy of Teratological Seedlings. IV.*

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Endemic Genera of Plants in their Relation to Others.

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With six Diagrams in the Text.

CONTENTS.

	PAGE
REVIEW OF THE HYPOTHESIS OF AGE AND AREA	493
ENDEMIC GENERA	501

REVIEW OF THE HYPOTHESIS OF AGE AND AREA.

INQUIRY is frequently made as to where a brief summary can be found of the ideas meant to be conveyed by what I have termed the hypothesis of Age and Area, and it will be well perhaps to commence this paper, which is the first of a new series in which somewhat larger issues will be treated, with such a summary. The new facts to which attention will be called below were discovered by aid of the hypothesis.

Examining on many occasions, from 1896 onwards, the volumes of the Flora of Ceylon (7, 8), so carefully worked up by Thwaites and Trimen, I gradually found, somewhat to my surprise, that the strictly local species confined to that island, or *endemic* species, as we usually call them, which are very numerous in Ceylon, showed on the average the smallest areas of distribution there, whether in the grand total or in individual families (cf. 10, p. 12). On the older view of the meaning of endemic species, which I then held, this seemed a very remarkable thing—that species which were generally looked upon as having been specially evolved to suit the local conditions should be so rare in those very conditions. If these species were specially adapted to Ceylon, therefore, it could not be to the general conditions of the island, but must be to strictly local conditions within its area. There was clearly no difference between island endemics and those of the mainland. Accordingly, still more remarkable did it seem when I came to study in detail the local distribution of these endemic species in Ceylon, and found that, as a rule, they were *not* confined each to one spot or small region characterized by some special local peculiarity in conditions, to suit

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which they might have been supposed to have evolved. Not only so, but such spots were frequently to be found with no local species upon them. Only about a quarter of the whole number were confined to single spots, and more than half of those were restricted to the tops of single mountains (12). The remaining three-quarters occupied areas of larger and larger size, and in diminishing numbers as one went up the scale. The three diagrams here reproduced give the ranges of the species in half of vol. i of Trimen's 'Flora', belonging to the three lowest of the six classes according to range into which he divided them. The VR (very rare) species are as a rule well localized, but the R (rare) and RR (rather rare), it will at once be observed, cover areas that *overlap* one another like the rings

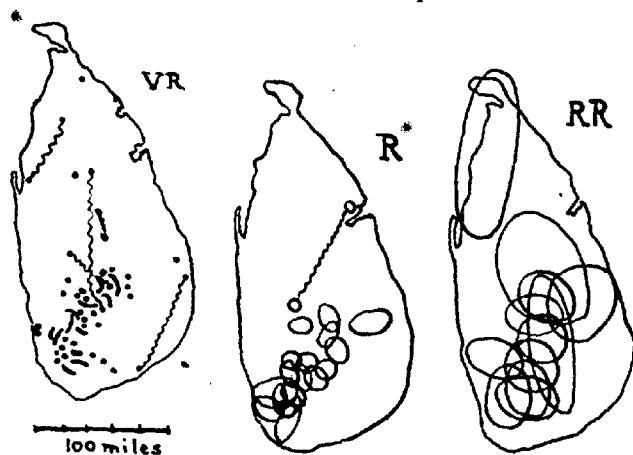


DIAGRAM 1. Distribution in Ceylon of the earlier VR, R, and RR species from Trimen's 'Flora'.

in a shirt of chain-mail. Now a little consideration will soon show that from the point of view of evolution to suit local conditions this is a very remarkable state of affairs.¹ If A and B grow in overlapping areas, both must be growing in the coincident portion, and what keeps A from growing into the rest of B's territory, B into A's? In reality the case is more complex, for if all the species were entered, there would be at least a dozen overlapping at any one point. It is all but inconceivable that local adaptation should be so minute as this, with soil essentially the same throughout, and the rainfall, &c., varying much from year to year. The species would have to be adapted to wide range in rainfall, and to very slight in a *combination* of other factors. It was clear that the old ideas of particular adaptation were quite untenable.

¹ It is of course obvious that if a species newly evolved does *not* suit local conditions it will not survive, but this is a different point of view from supposing it evolved *to* suit them.

Nor would the other popular theory, which equally survives to-day, satisfy the knowledge that I now had about local distribution. How could species be dying out in this remarkable chain-mail pattern, and why were there so many with small areas? Had one perhaps arrived in Ceylon just in time to see the dying out of a considerable flora? And why did so many choose mountain-tops as a last resort? If they had climbed from below, they must have plenty of adaptative capacity, and should be able to compete with the new-comers. Still more, why did each one or two choose a different mountain? One had not credited plants with the animal desire to die in solitude, and it was difficult to believe that the plains were once inhabited by different species at every few miles, whilst many mountains with endemics did not even rise direct from the plains, but from a high plateau.

Counting up all the species of the Ceylon flora, and dividing them into three groups—those endemic to Ceylon, those found only in Ceylon and South India, and those with a wider distribution abroad than this (which I termed *wides* for short)—I found (14, 15) the endemics to be graduated downwards from few of large distribution area to many of small (e.g. Common 90, Rare 192), and the *wides* in the other direction (e.g. Common 462, Rare 159), with the Ceylon-South India species intermediate. In other words, the average area occupied by an endemic was small, that by a Ceylon-South India species larger, and that by a wide the largest of all. A cursory examination of other floras showed me that their endemic species also behaved in the same way, occupying overlapping areas, and I was at last furnished with what seemed to me to be a much more feasible explanation of the distribution of species in general, and endemics in particular.

Having disposed, to my own satisfaction, of the notion that endemics were moribund species, I adopted the view that in Ceylon the *wides* were the first species (*on the whole*¹) to arrive, and had therefore on the whole occupied the largest areas. The Ceylon-South India species, on my view, must have arisen from them at points in general south of the middle of the peninsula, and would on the whole be younger in Ceylon than the *wides*, and therefore occupy lesser areas on the average. The Ceylon endemics would arise from the *wides* (or Ceylon-South Indians) in Ceylon, and would be the youngest, and on the average occupy the least areas. All the figures of course must be worked in averages, for an endemic of one group might be occupying a large area when the first wide of another arrived.

Such in brief was the gradual evolution in my mind, during twenty years, of the hypothesis which I have christened 'Age and Area', and which has been a good deal discussed of recent years. Before proceeding farther, I will quote the most recent expression of it, published in 1919 (22, p. 290):

¹ i. e. in any genus the wide would usually be the first to arrive.

"The area occupied at any given time, in any given country, by any group of allied species at least ten in number, depends chiefly, so long as conditions remain reasonably constant, upon the ages of the species of that group in that country, but may be enormously modified by the presence of barriers such as seas, rivers, mountains, changes of climate from one region to the next or other ecological boundaries, and the like, also by the action of man, and by other causes."

The Ceylon figures were strong evidence in favour of Age and Area, but as soon as possible I obtained confirmatory evidence by work upon the flora of New Zealand (16, also 17-24), where I was able to give actual longitudinal measurements of the areas where the species occurred, and where the results came out with much greater clearness. Still further work (19) on the Orchids of Jamaica, on *Callitris* (a Conifer) in Australia, and on the flora of the Sandwich Islands (both flowering plants and ferns, separately treated), also confirmed my conclusions. It will be well to quote here the distribution figures for New Zealand, as illustrating what I have said about the graduation of endemics one way and wide the other:

Range in N.Z.	Wides.	Endemics.
881-1080 miles	201	112
641-880 "	77	120
401-640 "	53	184
161-400 "	38	190
1-160 "	30 ¹	296

Many people who have tried to apply Age and Area to their own work have become dissatisfied with it because they have ignored the reservations which are given in the statement of the rule above, and we must now devote a few moments to their consideration. To begin with, it must never be applied to single species, but only to groups of at least ten allied forms. In order to cancel out the effects of differences among them in degree of local adaptation, of luck in transportation in the earlier rarer stages, and other more or less chance effects. In the second place, allied species must be taken, as in general they will belong to the same ecological type, and will react to their surroundings in more or less the same manner. It cannot be too often repeated that Age and Area does not as yet offer any means of distinguishing the comparative ages of differing ecological types, though I hope to show in a later paper that this is beginning to be possible to a slight extent. A group of Compositae may occupy the same area in the same country as a group of Dipterocarpaceae, but no one would suggest that they were of the same age within the country, though both groups obey the Age and Area rule. An area which might take the latter one thousand years to cover might be covered by the Compositae in one or two. Again, the conditions must remain reasonably constant. Any serious change might kill out some species. In essence, a change of conditions means

¹ Largely undoubted introductions of recent years.

a shifting of the ecological barriers, or the erection of such. Lastly, a fourth great reservation is expressed in what is said about barriers. The very great majority of species, for example, will be absolutely cut off from spreading by meeting a sea of any serious width, and the comparatively few species that may at times be able to cross may be easily appreciated by a study of Guppy's monumental labours on the subject. A broad river may act as a barrier to many, and probably will usually tend to delay spread, at any rate. The same is true of a high range of mountains, especially if they reach the snows, but here the barrier also as a rule becomes partly ecological, inasmuch as the climates on the two sides usually differ, to say nothing of the different climate at high levels. Other ecological boundaries than climatic changes are usually, in my opinion, too narrow to stop completely

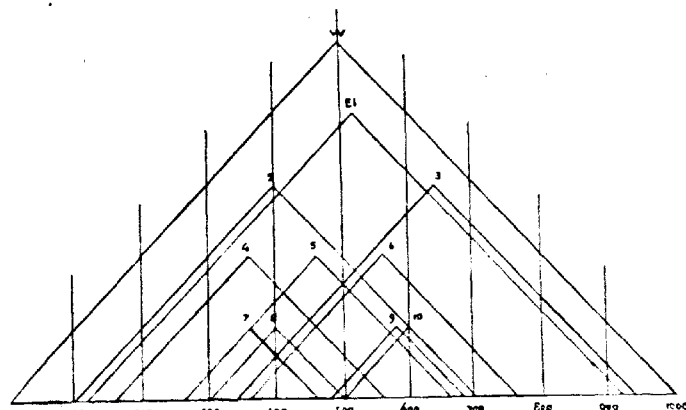


DIAGRAM 2.

the passage of species, though they may deflect, distort, or delay it; but the wording of the rule is framed so as to cover all possibilities. And finally, in modern times, as every one knows, man has done more than any of nature's agencies in the distribution of species about the globe, partly by intentional or accidental transport, partly by the great clearances, involving change of conditions, that he has made.

In studying the local distribution of species in New Zealand, where it was more exactly worked out than in Ceylon, I worked with the aid of a prediction that I made from a consideration of the bearings of Age and Area upon the subject (16, p. 442), that the number of endemic species in any genus would rise gradually to a maximum at or near the point or region where the genus entered New Zealand in the first place. The diagram here reproduced shows the way in which this occurs. A single wide (w) is supposed to enter New Zealand at the centre, and to follow

Age and Area with exactness in its distribution, as indicated by the right-angled triangle showing it gradually extending its area till at the same moment it reaches both north and south ends of New Zealand. Casually, it or some of its derivatives give rise to ten endemics (E, 1 to 10), which

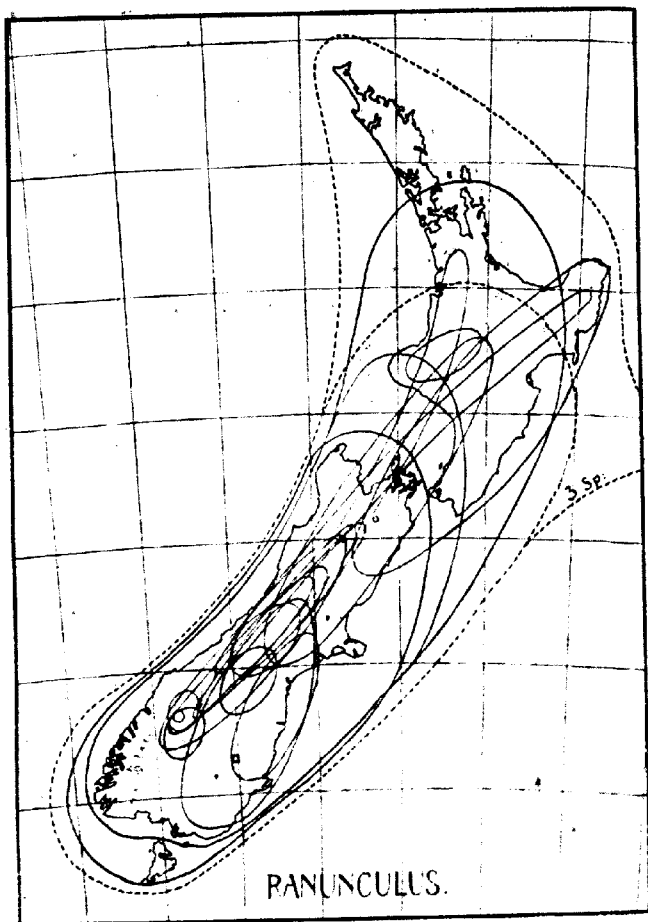


DIAGRAM 3. Wides dotted; extension N. includes Kermadec, E. Chatham.

spread in the same way. If a vertical line be drawn at each tenth of the total distance from north to south, and the number of endemics in each zone counted, they will give a curve rising to a maximum at or near the middle (sometimes two maxima). Here for example the figures run 0, 3, 5, 8, 9, 8, 7, 3, 2, 2. On examination, I found this maximum exhibited by every

genus in the flora that had more than one species, and a planning of the areas occupied by the different species resulted in giving for all the larger genera such maps as that for *Ranunculus* here reproduced, which shows clearly the massing of the species near the middle of the South Island. Examination of all the genera showed that these maxima occurred, not casually all over New Zealand, but in masses, especially at the far north, at the centre, and at the point where they show in *Ranunculus*, rather to the south of the middle of the South Island. Upon these results I have based my contention that the flora of New Zealand has been the result (24, p. 475) of four separate invasions—one from Indo-Malaya direct (northern invasion, reaching New Zealand at the far north, and when added together giving the

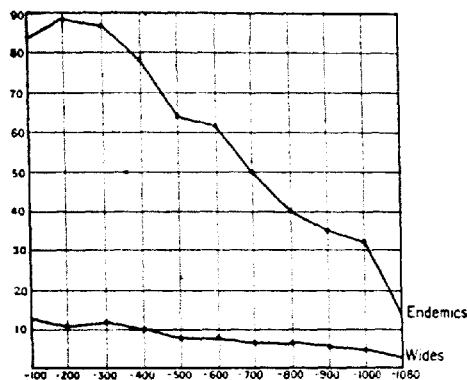


DIAGRAM 4. Northern invasion: vertical figures are numbers of species, horizontal the distances in miles from North Cape of New Zealand.

curves in Diagram 4, which shows the numbers of wides and endemics at each successive zone of one hundred miles, working south), one from Polynesia by way of the Kermadec Islands, one from Australia (western or central invasion), and one from the south (southern invasion, curves given in Diagram 5, with maxima¹ at 800-900 miles south of the North Cape, i.e. rather south of the middle of the South Island.

Results were now flowing in so easily and naturally that I became a firm believer in the truth of my hypothesis, and applied it to predictions of what would or would not be found in certain places or under certain conditions. Some of these predictions were simple tests, as when I predicted (21, p. 27) nearly half the species that would be found upon Stewart Island from a knowledge of the flora of New Zealand, or all but fourteen of those

¹ The double curve given for wides is explained thus: a few species of some of the genera are evidently northern, and die out as one goes south. The upper curve includes these, the lower and more correct curve excludes them. The narrowing distance between the curves shows the way in which they die out to the south.

upon the Chathams (22, p. 288); but most have been devoted to the discovery of new facts, or still more to the regrouping and marshalling of old and incompletely known or understood facts. Over ninety predictions

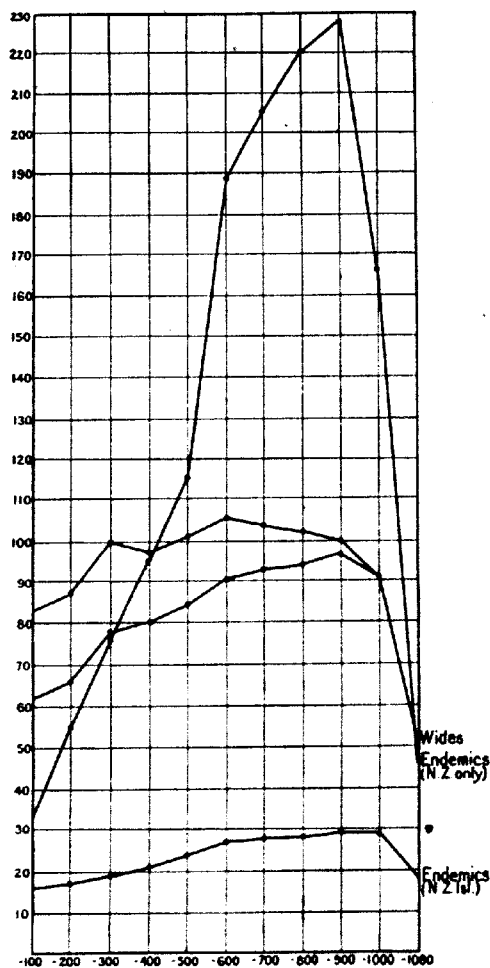


DIAGRAM 5. Southern invasion: figures as in northern invasion.

have now been made, and verified by a subsequent study of the facts, but it would lead too far to go into further details. As I am sometimes accused of making the prediction subsequently to the knowledge of the facts, I may perhaps be allowed to state that this is not the case, but that I have con-

sidered each case carefully, and thought out what should be found if Age and Area were true, and then have subsequently verified it by an examination of the facts, in all cases finding that my prediction was correct. In the case about to be described, for example, I first thought out the predictions, and then sat down to count and classify genera for six weeks, in perfect confidence that the result would come out in accordance with my anticipations—as in fact it did. The present era in the history of the world and its peopling with plants seems to be an era governed mainly by Age and Area, which has determined in broad outline the distribution of plants about the world, leaving to ecology the settlement of the details.

ENDEMIC GENERA.

In this paper I propose to attempt to extend the applicability of Age and Area, which hitherto has been confined to the plants of one country, and to species only, with occasional incidental references to genera. It will of course be understood that the larger the area and the number of plants dealt with, the less clearly does its operation show in detail, but I venture to hope that it will be admitted, after consideration of what is set forth below, that it does hold in a wide and general manner for all flowering plants and for the whole world.

I shall deal principally with what are called endemic genera, confined to one island, one mountain chain, or other restricted locality. In one of my first papers (15, p. 5) I pointed out an obvious deduction to be drawn from an acceptance of Age and Area, viz. that as a rule endemic species confined to small areas are in reality species in the earlier stages of spreading about the globe, and given time enough and absence of barriers they might ultimately be found covering large areas. The corollary to this, that endemic genera are similarly young genera, I have left to be inferred, but it will be elaborated below.

The expression of this view has met with considerable opposition, for it involves a break with the opinion that has so long held the field, that endemic forms, whether species or genera, represent some kind of losers in the struggle for existence, and that the regions characterized by their presence are therefore to be regarded as a kind of refuges for the destitute, where, on account of the smaller number of species, there was less keen competition, and these forms had been able to survive it. It is thus expressed in a recent paper (3, p. 215). 'Very many endemics owe their limited distribution to the circumstance that they are remnants of comparatively unsuccessful types which have been exterminated elsewhere, and

which even in these isolated floras are waging a losing fight against more vigorous and adaptable new-comers.'

This explanation has always been unsatisfactory to me since I began to study *in situ* the numerous endemic forms of Ceylon, and a great argument against it, which has always been passed over and left unanswered by its supporters, is the fact that endemic species usually occupy continuous areas. In other words, from the point of view of the current explanation, the endemics have not retired in confusion, but have kept their formation. Other arguments which also turned up in the course of my work confirmed me in this disbelief of the usual explanation. For example, I found it very difficult to reconcile with the idea that endemics were in general moribund the fact that in a single country there was a regular graduation of endemics upwards from many of small area of distribution to few of large, and of species of wider distribution than endemic in the opposite direction (cf. tables in 14, p. 310; 15, p. 3; 16, p. 449; 19, pp. 336, 338, 344). Again, practically all the endemics of a country, if they possess more than one or two species, show graduated maps like that given for *Ranunculus* in New Zealand, and the same is the case whether they have or have not widely distributed species of the same genus beside them. Another awkward point for the supporter of generally moribund endemics (i. e. other than a comparatively few, too small in number to affect the figures) is the fact that in the far outlying islands round New Zealand (Chathams, &c.) the more widely distributed species there are, the more endemics (18, p. 331, Table III), and the wides are more numerous in proportion (figures in 20, bottom of p. 352). Further, the endemics of New Zealand and Ceylon are most numerous where there are most wides, least where there are fewest, which is not at all what one would expect if the endemics are moribund species. The comparatively few endemics found near the outer ends of New Zealand range over a much greater area than do those in the middle (cf. the map of *Ranunculus* above, and table in 16, p. 448). Yet another great difficulty, from the old point of view, is the fact that species endemic to New Zealand and its immediately outlying islands (Kermadecs, Chathams, Aucklands) are on the average more widespread in New Zealand than the species common to New Zealand and the outside world, but *not* found on these little islands, while the species found in the outside world, and occurring also in New Zealand *and* these islands, are the most widespread in New Zealand of all. Fern endemics, which must on the whole be older than angiosperm endemics, occupy more area than the latter. Lastly, and perhaps most important of all, the endemic species belong principally to the *large*, or what on the old view are considered the successful genera, and much less, even in proportion, to the small.

All these, and many other arguments which might be brought forward (cf. 20, p. 352), rendered it impossible for me any longer to adhere to the

view that endemics were moribunds—at least in any serious proportion—but it is only during the last seven years that I have been able to bring proofs for what seems to me a more satisfactory explanation, viz. that they represent different early stages on the way *upwards*, not downwards. One cannot imagine species dying out in the regularly graduated way shown in the map of *Ranunculus* above, but if one imagine that these are new species formed in successive order, and just in the early stages of dispersal, one arrives at a perfectly simple explanation, which completely fits the facts so far as we know them.

In view of the incisive figures which have been set forth in recent papers, a number of botanists are now beginning to admit that endemic species of small area of distribution are really young species, or at least have been formed *in situ*, but few are as yet prepared to admit this for endemic genera, and most people continue to regard them as survivals.

If my view be the correct one to take, then it is clear that there is no difference between endemic genera and species, and others that occupy larger areas, except that in general they are younger; so that while one may call *Coleus elongatus* endemic to the summit of Ritigala in Ceylon, or the genus *Carpodetus* endemic to New Zealand (it has one species, reaching to both ends of New Zealand), one may with equal justice talk about *Hottonia palustris* as endemic to temperate Europe and Asia, or the genus *Senecio* as endemic to the world. My aim in the present paper is to show that this is the more correct view to take, and that there is no appreciable difference but age.

So firmly has the old view, that endemics are relics of old floras, held sway, that it never seems to have occurred to any botanist to try the simple test whose results I am about to set forth, and yet this test might have been made at any time in the last thirty years. Although it was suggested by a consideration of Age and Area, the results here set forth are simple facts which may be considered purely on their merits, without reference to any hypothesis whatsoever.

One may test this question, whether endemic genera are or are not in general relics, in a very simple way, by making a prediction which shall at the same time be a crucial test, inasmuch as the result must be different on the two hypotheses, and then verifying it by comparison with the actual fact. The prediction, however, being as to what, in a broad general way, will be found upon the islands of the world, involves various complications.

In the first place, if the islands have received their floras by casual oversea transport, it is clearly all but impossible to predict what will be found upon them. If a successful prediction is made, therefore, the fact speaks very strongly in favour of their having (in the mass) received their floras by land, not necessarily continuous, but with no very large gaps. If

any really large islands received by water, the prediction would probably break down.

If endemic genera are survivals, one cannot predict to what families they will belong, except that it would seem highly improbable that they should belong *mainly* to the large and what are usually called the 'successful' families, like Compositae or Rubiaceae. It would seem more probable that they would show a tendency, at any rate, to belong to families that are small, or of broken distribution, such as we have been accustomed to consider as unsuccessful and more or less moribund. In any case, one would expect some marked differences in composition of the list from that of the mainland.

If then, examining the endemic genera of islands, we find them to be a miscellaneous assortment, we may imagine either that the islands received their floras mainly by casual oversea transport, or that the endemics represent survivals, and if we also find that they show a distinct tendency to belong to the small and broken families, we may then with a fair degree of probability accept the second of these suppositions.

If, on the other hand, endemic genera be young genera in the earlier stages of spreading, as is the case if we accept the hypothesis of Age and Area, we shall expect them (provided of course that the connexion was mainly by land) to appear in families in proportions not dissimilar to the proportions of genera appearing in those families at the present time. There should be among them many Compositae and Rubiaceae, few Magnoliaceae or Myristicaceae, more Melastomaceae than Ranunculaceae, and so on.

But we may go farther than this. As I have already pointed out (21, p. 34), if Age and Area holds, then on the whole a large family will be older than a small one of the same circle of affinity. And as the islands were mostly cut off from the mainland at a remote period, the families that reach them will on the whole be the older, that is to say, rather the larger than the smaller. And as the larger would be more likely to get to them first (as being on the whole older than the smaller), they will tend to have rather more genera in proportion upon the islands. One will therefore expect the proportion of endemic genera upon the islands to be if anything rather greater in the large than in the small families.

Other factors will also come in to influence the result. Some families, like Compositae or Orchidaceae, may be well able to travel across wide stretches of water, and may thus be better represented than their age would indicate. If herbs are younger, as there is good reason to suppose (5), we shall expect many herbaceous families, even though they may be large, to be badly represented, and so on.

This second prediction, then, if it prove successful, will on the whole show that the bulk of the connexion to the islands at the time they

received their floras was by land and not by casual oversea transport, and it will also show that the distribution accorded in general with the rule of Age and Area, and that the endemic genera in general are young genera in their early stages, and not survivals.

In order to arrive at a result which cannot be the subject of cavil on account of incompleteness, I have included in the present paper all the endemic genera of all the islands in the world, amounting to 1,582, or 12.6 per cent. of the 12,517 in the world. I have taken them from my Dictionary (4th ed., 1919), thus including many that would generally be merged in others, but at least getting a complete list of all that are at all frequently considered as separate genera.

The three great groups of islands in the world are all in the tropics, and one must bear this in mind in considering the composition of their lists of genera. A counting of the genera in each of these three groups—(1) the Indo-Malayan Islands, including Ceylon, the Malay Archipelago, Polynesia, New Caledonia, &c.; (2) the African Islands, including Madagascar, the Mascarenes, Socotra, &c.; (3) the American Islands, including the West Indies and Galapagos—soon shows that in all much the same families stand at the top of the list of genera. The following table shows the first ten families in each case, with the number of endemic genera belonging to them:

1. <i>Indomal. Islands.</i>	2. <i>African Islands.</i>	3. <i>American Islands.</i>
1. *†Orchidaceae 79	*†Rubiaceae 29	*†Rubiaceae 25
2. *†Rubiaceae 67	* Palmaceae 27	*†Compositae 20
3. * Palmaceae 41	*†Asclepiadaceae 15	*†Euphorbiaceae 13
4. *†Euphorbiaceae 38	*†Acanthaceae 24	*†Leguminosae 13
5. Araliaceae 26	*†Compositae 22	* Palmaceae 13
6. Melastomaceae 23	*†Orchidaceae 19	*†Orchidaceae 12
7. Anonaceae 19	*†Leguminosae 15	*†Acanthaceae 8
8. *†Leguminosae 19	Sapindaceae 14	*†Asclepiadaceae 7
9. Sapindaceae 19	*†Euphorbiaceae 13	Apocynaceae 6
10. Apocynaceae 17	Bignoniaceae 10	*†Gramineae 6

* In all three lists.

† In the first ten largest families of the world.

This table, incomplete though it be, does not offer any suggestion that the endemics of the islands are survivals. There are only seven families in all that do not come into the first ten in the world: of these Palmaceae, Melastomaceae, Sapindaceae, and Apocynaceae are found in the second ten, and of the rest Bignoniaceae are twenty-second in the world with 119 genera, Anonaceae twenty-sixth with 108, and Araliaceae thirty-ninth with 81.

The enormous majority of the endemic genera of islands belong to the three island groups just mentioned, and when one adds up the grand total for all islands in the world one arrives at the following table:

		<i>Gen. on Islands.</i>	<i>Gen. in the World.</i>			<i>Gen. on Islands.</i>	<i>Gen. in the World.</i>
1. Rubiaceae	(5)	124	506	21. Icacinaceae		18	63
2. Orchidaceae	(2)	120	666	22. Flacourtiaceae		17	104
3. Palmaeae		86	216	23. Menispermaceae		17	96
4. Compositae	(1)	81	1,143	24. Zingiberaceae		17	60
5. Euphorbiaceae	(7)	65	326	25. Bignoniaceae		16	119
6. Leguminosae	(3)	56	645	26. Liliaceae		16	254
7. Asclepiadaceae	(6)	47	347	27. Myrtaceae		15	90
8. Acanthaceae	(10)	45	266	28. Verbenaceae		15	89
9. Gramineae	(4)	43	506	29. Monimiaceae		14	34
10. Melastomaceae		36	186	30. Umbelliferae	(8)	14	325
		703	4,807			159	1,234
11. Sapindaceae		34	156	31. Urticaceae		14	50
12. Araliaceae		33	81	32. Labiatae		13	197
13. Apocynaceae		29	198	33. Meliaceae		13	68
14. Rutaceae		22	144	34. Ericaceae		12	119
15. Saxifragaceae		22	96	35. Guttiferae		12	54
16. Araceae		21	131	36. Myrsinaceae		12	41
17. Sapotaceae		21	81	37. Boraginaceae		11	114
18. Anonaceae		20	108	38. Campanulaceae		11	73
19. Scrophulariaceae		20	241	39. Cruciferae	(9)	11	289
20. Gesneriaceae		19	120	40. Cunoniaceae		11	30
		241	1,356			120	1,035

The first ten in the world in order of size are marked in brackets after the name.

These first 40 families, in order of number of endemic genera upon islands, include 31 of the first 40 families in the world in order of size. The 9 others included are Campanulaceae (forty-third in the world), Meliaceae (forty-seventh), Icacinaceae (fiftieth), Zingiberaceae (fifty-second), Guttiferae (fifty-ninth), Urticaceae (sixty-second), Myrsinaceae (sixty-eighth), Monimiaceae (seventy-eighth), and Cunoniaceae (eighty-fifth). The corresponding nine in the world list that are missing are (in order of size) Rosaceae (eighteenth), Cucurbitaceae (twenty-fifth), Cyperaceae (twenty-seventh), Solanaceae (thirty-first), Amaryllidaceae (thirty-second), Caryophyllaceae (thirty-third), Chenopodiaceae (thirty-sixth), Anacardiaceae (thirty-seventh), and Gentianaceae (thirty-eighth), practically all herbaceous or shrubby families.

These first 40 families include 1,223 out of 1,582 endemic genera of islands, or 77 per cent. of the total, the remaining 23 per cent. being comprised in the other 110 (of the 252 remaining) families in the world which possess endemic genera upon islands. These families are:

With 11 genera endemic upon islands, Tiliaceae; with 10, Celastraceae, Malvaceae, Moraceae; with 9, Anacardiaceae, Lauraceae, Thymelaeaceae; with 8, Chlaenaceae, Coniferae, Loranthaceae, Proteaceae, Rhizophoraceae, Rosaceae, Solanaceae, Sterculiaceae; with 7, Cucurbitaceae, Loganiaceae, Rhamnaceae, Simarubaceae; with 6, Caryophyllaceae, Dipterocarpaceae, Gentianaceae, Hamamelidaceae, Olacaceae, Theaceae; with 5, Bombacaceae, Burseraceae, Burmanniaceae, Convolvulaceae, Cornaceae, Elaeocarpaceae,

Malpighiaceae, Violaceae; with 4, Amarantaceae, Ochnaceae, Piperaceae; with 3, Caprifoliaceae, Cyperaceae, Dilleniaceae, Lecythidaceae, Lythraceae, Marantaceae, Nyctaginaceae, Passifloraceae; with 2, Amaryllidaceae, Aquifoliaceae, Balanophoraceae, Balanopsidaceae, Begoniaceae, Bromeliaceae, Dioscoreaceae, Epacridaceae, Geraniaceae, Globulariaceae, Linaceae, Magnoliaceae, Orobanchaceae, Papaveraceae, Polygonaceae, Portulacaceae, Rafflesiaceae, Ranunculaceae, Staphyleaceae, Turneraceae, Winteranaceae; with 1, Aizoaceae, Berberidaceae, Cactaceae, Cercidiphyllaceae, Chloranthaceae, Cnecoraceae, Cochlospermaceae, Combretaceae, Commelinaceae, Connaraceae, Corynocarpaceae, Crassulaceae, Cycadaceae, Datisceae, Diapensiaceae, Ebenaceae, Flagellariaceae, Frankeniaceae, Gonyostilaceae, Goodeniaceae, Hippocrateaceae, Hydrophyllaceae, Lactoridaceae, Loasaceae, Myoporaceae, Myristicaceae, Opiliaceae, Oxalidaceae, Pandanaceae, Pedaliaceae, Philydraceae, Phytolaccaceae, Podostemaceae, Polygalaceae, Primulaceae, Sabiaceae, Santalaceae, Sonneratiaceae, Stylidiaceae, Styraceae, Theophrastaceae, Triuridaceae, Trochodendraceae, Ulmaceae, Vitaceae. Grand total 150 fams., 1,582 gen.

It is clear from a cursory inspection of this table, that the endemic genera of islands belong to the families in rough proportion to the actual totals of genera contained in them, though, as we shall see in detail in a later paper, a number of herbaceous families like Compositae, Cruciferae, Caryophyllaceae, Liliaceae, Umbelliferae, Chenopodiaceae, &c., are distinctly low in the scale of island genera, a fact indicating that they are probably on the whole younger (cf. 5).

The families that are missing in the list of island genera also bear out the same conclusion. They are 142 in all, out of a total of 292, or nearly half, are mostly families with small totals of genera in the world, and are chiefly herbaceous, or undershrubs. They form the following list:

Chenopodiaceae	with 86 genera in the world
Iridaceae	.. 68 "
Onagraceae	.. 51 "
Capparidaceae	.. 49 "
Zygophyllaceae	.. 31 "
Oleaceae	.. 27 "
Restionaceae	.. 25 "
Polemoniaceae	.. 23 "
Haemodioraceae	.. 21 "
Hydrocharidaceae	.. 21 "

With 7 families with 11 to 18 genera
.. 24 6 to 10 ..
.. 49 2 to 5 ..
.. 52 1 genus

One may compare this list with the list of 150 families that contain the endemic genera of islands, and get the following table:

Willis.—Endemic Genera of

<i>Fams. with endemic genera on islands.</i>			<i>Fams. without</i>
With	1 genus	3 *	52
"	2-5 genera	19	49
"	6-10 "	12	24
"	11-30 "	36	12
"	31-50 "	21	2
"	51-80 "	19	2
"	81-150 "	23	1
"	over 150 "	17	—

which shows that the families with no island endemic genera are crowded towards the bottom of the list. Or yet again, one may take the total of genera in the families of the two lists, when one finds that the 150 families in the first list, that contain endemic genera on islands, contain in all 11,627 genera, an average of 77 per family, while the 142 in the second list contain in all 890, or an average of 6 per family.

It is quite clear that the facts do not bear out any prophecy based on the idea that endemics are moribund. The small families, and families of disconnected distribution, show no unusual prominence, but are on the contrary very badly represented.

But the final proof against the moribund nature of endemic genera as a whole, both for islands and for other places in which they abound, such as South Africa or West Australia, is given by adding up all the genera found *only* in

1. The islands of the World.
2. West Australia, South Africa, and Brazil, all areas rich in endemic forms, and among them showing much variety in conditions, with forest, campo, and desert.
3. Australia, Africa, and South America.
4. The World.

Just as in the case of the groups of islands, the individual families vary in their arrangement amongst themselves in the various lists; indeed, were it not so, the fact would be perfectly astonishing. But it is generally found that any one family (unless herbaceous) is not *very* far apart in its situations in the different lists, and if we add the families together in groups of ten, local variation will be quite sufficiently covered. Taking as a standard the groups of ten into which the world list divides, and adding together in each of the other lists the genera found in the *same* ten families, we arrive at the table on p. 509.

The figures in this table are very striking, and bear out in a very complete manner the prediction based upon the supposition that endemic genera cannot be survivals, except in rare cases. The percentage numbers are so close together in the different columns that the largest difference of all is that in the second line, between 18.0 for islands and 14.6 for Australia, Africa, and South America combined. It is clear in the first place that the islands, with few exceptions, and those almost certainly not the large

islands like Madagascar, New Zealand, or Borneo, must have been connected with the mainland at the time when they received the bulk of their flora, though the connexion need not have been absolutely continuous, but might have been interrupted by narrow straits. And it is also, I think, clear in the second place that endemic genera, with few exceptions, are really young genera in the earlier stages of dispersal. Just as it was all but inconceivable that endemic species, which showed as 'wheels within wheels', as in the map of *Ranunculus* in New Zealand given above, should be dying out in so regular a manner, so here it is extraordinarily hard to imagine that the genera can be survivals, and yet appear in a regular proportion to the totals of genera in their various groups.

<i>Tens of Fams.</i>	<i>World.</i>		<i>Austr. &c.</i>		<i>W. Austr. &c.</i>		<i>Islands.</i>	
		%		%		%		%
1.	5,019	40.1	1,579	39.1	459	40.5	606	38.3
2.	1,868	14.9	592	14.6	179	15.5	285	18.0
3.	1,094	8.7	360	8.9	86	7.6	144	9.1
4.	874	6.9	325	8.0	78	6.8	115	7.2
5.	695	5.5	271	6.7	75	6.6	83	5.2
6.	561	4.4	216	5.3	57	5.0	82	5.1
7.	456	3.6	83	2.0	19	1.6	55	3.4
8.	355	2.8	111	2.7	30	2.6	48	3.0
9.	299	2.3	99	2.4	24	2.1	29	1.8
10.	233	1.8	79	1.9	29	2.5	37	2.3
Total	11,451	91.4	3,715	92.1	1,033	91.1	1,484	93.7
11 to 20	919	7.3	278	6.8	90	7.9	86	5.4
21 to 29.2	147	1.1	38	0.94	10	0.88	12	0.75
Grand Total	12,517		4,031		1,133		1,582	

The percentages may be plotted as curves (Diagram 6, p. 510), when their agreement is seen in a still more striking manner.

These curves are very remarkable in their close coincidence, and after sight of them it is difficult any longer to maintain the position that endemic genera in general are survivals of old floras. Survivals are not likely to be nicely graduated in proportion to the size of the groups of families to which they belong.

The first and principal part of the prediction is thus fully borne out, and comparison shows with equal clearness that the proportional representation of the different families among the endemic genera of islands decreases as one goes down the scale. There are 292 families of Spermaphyta in the world, and the first 100 of these have island endemic genera in 92, the genera being 12.9 per cent. of the total genera in the families. The intermediate 92 families are represented in the islands by 45 only, with 9.28 per cent. of their genera, while the final 100 are represented only by 13 families endemic with 8.72 per cent. of their genera. This bears out the second part of the prediction, whose complete success may be taken to mean that in general, with few and comparatively insignificant exceptions, the islands were united to the mainland at the time they received their floras.

and that they received these floras, in accordance with the principles of Age and Area, the endemic genera in them being in general simply the younger genera that have not yet spread very far.

A little more must be said to make clear the position with regard to relic endemics, and possibility of genuinely oceanic islands. Nothing that has been said above is to be read as denying that either of these may exist, but it is clear from the figures that they are unimportant as compared with the endemics which are not relics, and the islands which are not truly oceanic, but have been peopled with plants by land connexions. There is

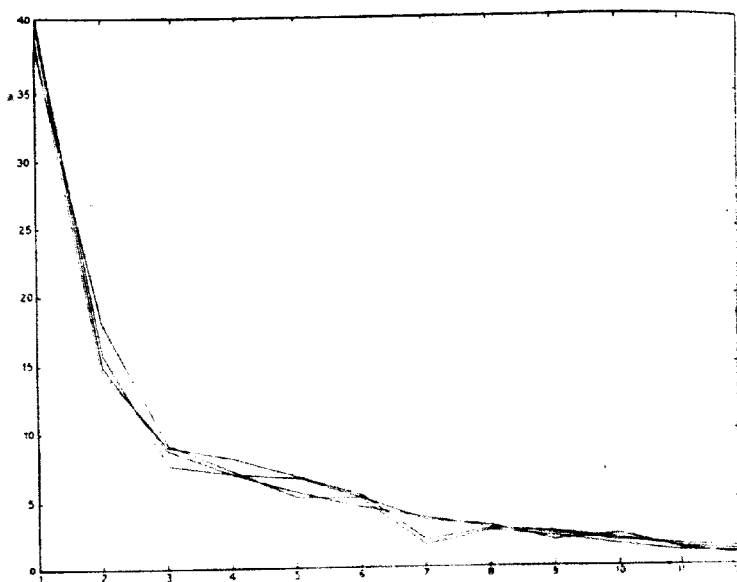


DIAGRAM 6.

an appreciable sprinkling of plants, such as *Ginkgo* for example, which are now confined to small areas, and which we know from geological evidence to have been formerly widespread; but these are but few and far between in the grand total of endemics. In the mass, it is no longer possible to look upon endemic genera as being survivals; evidence must be definitely brought up in each individual case in which it is desired to prove that an endemic is a survival. And the same general statement is true with regard to the oceanic nature of islands. It is not possible to regard islands, in the bulk, as having received their floras across the present existing wide stretches of water; they must in general have received their floras by way of land con-

nexions, but that statement does not exclude the possibility that some few may have been oceanic, such for instance as the Sandwich Islands, or St. Helena. These far outlying islands have so few endemic genera that they do not appreciably affect the totals.

Confirmatory evidence may be obtained in various ways. One may, for example, point out that since many families have been long enough upon islands to have given rise there to endemic genera, these families must be very old. But now, to have reached both the Old and the New Worlds in the course of its dispersal, a family must also be very old. One will therefore expect that a very great proportion of those families that are represented upon islands by endemic genera will also reach both worlds. Examining the facts, one finds that 5 families with endemic genera upon islands are themselves endemic there. Of the remaining 145, no fewer than 131 (90 per cent.) reach both Old and New Worlds, while of the other 142 families that have no endemic genera upon islands, only 75 (52 per cent.) occur in both land masses.

Or again, on the whole the islands that are farthest out from the mainland should have almost solely the very oldest families (of the nearest mainland) in their various affinity circles, and we have seen that these are usually also the largest families. Thus, on the whole, the endemic genera of the far out islands should belong to larger families, averaging larger than the islands as a whole, though these should average larger than those of the mainland. We may test this on the flora of Madagascar. This island has endemic genera in 80 families, while the islands of the world have them in 150. Of the 70 families thus missing, no fewer than 57 belong to the 75 smaller, and only 13 to the 75 larger families. In New Zealand the proportion of families with endemic genera is 16 in the first 75 larger to 4 in the 75 smaller families, while in the Sandwich Islands the proportion is 13 to 1. The prediction is thus fully borne out, and it is confirmed by the fact that the bulk of the small families occur only on the nearer islands. Or reference may be made to the comparison between the floras of New Zealand and the outlying Chatham Islands (22, p. 287).

If the island endemic genera were really survivals, they should, in virtue of their total number of 1,582, or 12.6 per cent. of the genera of the world, contain at least that percentage of the monotypic families, but in fact, of the 55 such families, they contain only 3. Of the other 52, 12 are found in both hemispheres, 6 are palaeotropical, 6 neotropical, 6 in Africa, and smaller numbers elsewhere.

The oldest families of all will in general have reached most islands. One will therefore expect the bulk of the endemic genera to belong to families that reach many islands. This is easily found to be the case, so there is no need to go into detail, but give simply one instance. The West Indies have 195 endemic genera in 43 families that also occur in the islands

of Indo-Malaya, and only 19 in the 16 families that do not; they have 187 in 39 families that occur on the African islands, and 27 in 20 families that do not.

Postscript. Since writing the above, I have been working at the endemic genera of N. temperate America, and I find, as I shall show in a later paper, that the misunderstanding between Prof. Sinnott and myself is due to the fact that there *are* many relics there, while there are not in the tropics, to which most of the islands belong, nor in Europe. Each of us has reasoned from the territory that he has most closely examined.

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And cf. List of Literature in 20.

The Testimony of the Endemic Species of the Canary Islands in Favour of the Age and Area Theory of Dr. Willis.

BY

H. B. GUPPY.

MANY botanists of eminence have interested themselves in the Canary flora, and much has been written about it. An abundance of materials is, therefore, at our disposal, enabling us to appreciate the methods followed in dealing with the endemic species and the standpoints adopted with regard to the problem of their origin. It is to these two subjects that the following remarks will be entirely devoted.

Like most archipelagos that with other adjacent groups of islands go to form a separate floral region, the Canary Islands display a double endemism, the endemism peculiar to the group and the endemism that it shares as a member of the floral region. There are the endemic species peculiar to the archipelago, the Canarian proper, and there are the endemic species which it holds in common with the other groups of the Macaronesian floral region, the Azores, the Madeiras, and the Cape Verde Islands. The true Canarian endemic species number about 400, whilst the Macaronesian species, which occur in other Macaronesian groups but are not known from any other floral region, number about fifty. As far as my re-earches indicate, this di-proportion is typical of insular floral regions.

The Macaronesian endemics are far more generally distributed over the Canary Islands than are the Canarian proper. From the materials supplied by Christ and by Pitard and Proust it appears that whilst only 8 per cent. or 10 per cent. of the Macaronesian endemic species have been recorded from one island only, between 60 per cent. and 65 per cent. of the Canarian endemics are only known from single islands. On the average the Macaronesian endemics occur in three or four islands (3.5), but the Canarian are found in only one or two (1.7).

The following tables have been prepared by the writer from the work of Pitard and Proust :

[Annals of Botany, Vol. XXXV. No. CXL. October, 1921.]

A.

Distribution in the Canary Islands of *Macaronesian* endemic species—that is, of species restricted to that floral region, but shared by the Canaries with other Macaronesian groups (Azores, Madeiras, Cape Verdes). There are seven principal islands.

<i>Number of Islands.</i>	<i>Number of Species.</i>	<i>%.</i>
One	4	8
Two	9	18
Three	12	24
Four	14	28
Five	6	12
Six	3	6
Seven or more	2	4
	<hr/> 50	<hr/> 100

B.

Distribution in the Canary Islands of *Canarian* endemic species—that is, of species confined to that group as distinct from the Macaronesian endemics which characterize Macaronesia as a whole.

<i>Number of Islands.</i>	<i>Number of Species.</i>	<i>%.</i>
One	248	62
Two	73	18
Three	35	9
Four	28	7
Five	8	2
Six or more	8	2
	<hr/> 400	<hr/> 100

There can, therefore, be no doubt that in the Canaries the endemic species that also occur outside the archipelago in the other Macaronesian groups have a much wider distribution than those restricted to the islands, range in the group going with range in the whole floral region, as Dr. Willis has established for New Zealand. We will now ascertain how botanists have explained the origin of the two kinds of endemic species. In the first place we will deal with the Canarian endemics proper. Hooker had long been interested in the floral history of the group. He gave it a prominent place in his *Lecture on Insular Floras* in 1866, and twelve years later he gave his matured views in the appendix to the joint work by Ball and himself on '*Marocco and the Great Atlas*' (p. 417). For him the endemic species peculiarly Canarian came into being as derivatives of parents of Mediterranean type that reached the islands long ago. As an example of the prevailing methods and 'generally accepted views' among systematists in the latter half of last century Hooker's remarks have great importance in connexion with the subject of this paper, and they are here quoted in full:

'The wonderful development in the Canaries of endemic species, belonging for the most part to Mediterranean types, points to the very early

introduction of the parent forms of these, and the long isolation both of the Archipelago and its separate islets. It is in accordance with generally accepted views to assume that the endemic species of each genus have been derived from parent forms originally introduced into one or more of the islets; and that as the descendants of these species spread over the Archipelago they were exposed to different conditions in each islet, resulting in their varying, and in the segregation and conservation of different local varieties each in its own insular birthplace; a supposition, which is in accordance with the fact that those endemic species are really very local, many being confined to a single islet.'

But Hooker held quite a different view for the origin of the Macaronesian species, and especially for the most typical of them, those of the Laurel Woods. Whilst regarding the mass of the non-endemic species of the Canaries as Mediterranean plants, and the mass of the true Canarian endemics as derivatives of yet earlier Mediterranean types, he recognized a great break in the floral history of the group when, on taking a step farther back, he came to handle the Macaronesian species. Here he found the wreck of an ancient continental flora which, having been expelled from the continent through secular changes of climate, had 'been preserved in the more equable climate and more protected area of the Atlantic Islands'. This view, which was elaborated in his Lecture in 1866, was restated in his book on Morocco (pp. 417, 419). It was based on the discovery of plants in the Tertiary beds of Southern Europe, closely allied to or identical with living Macaronesian species.

The trend of the later evidence indicates that the Canaries and the Macaronesian groups generally are by no means alone in this respect, and that islands have often been sanctuaries for the survivors of continental floras that have passed away. However this may be, the view of Hooker, that the remains of an extinct European Tertiary flora still survive in the Macaronesian Islands, was combated by Grisebach in his 'Die Vegetation der Erde' (1872), but, as Engler has shown, on quite insufficient grounds. It was strongly supported and extended by Engler himself in his 'Versuch einer Entwicklungsgeschichte der Pflanzenwelt' (1879-82, i. 74), and had in the meanwhile been confirmed by later geological discoveries, notably those of Saporta and Marion (1876). Engler held to the position tenaciously, and remarked that 'even if we do not allow that the existing Macaronesian species are but slightly altered forms of species which lived in Europe in Tertiary times, we have sufficient other grounds for the belief that the endemic Macaronesian flora dates in great part from the Tertiary age and that the insular conditions have contributed to its preservation'. Drude, in his 'Handbuch der Pflanzengeographie' (1890), deals on similar lines with the Tertiary character of a portion of the endemic element of the Canarian flora.

This brings us to another stage in the argument. We now perceive that whilst the species peculiar to and characteristic of the Macaronesian floral region have a much wider range in the Canaries than the purely Canarian endemics, they are also far older. Whilst the Macaronesian plants are remnants of old Tertiary forests of Southern Europe and North Africa, and represent types that have disappeared from the continent, the purely Canarian endemics belong to types still predominant in the Mediterranean region. The Macaronesian endemics are true 'relicts' and are widely spread over the Canarian archipelago; whilst the purely Canarian species are of recent and local origin, and are for the most part limited to single islands. As already implied, the species peculiar to the Canaries are eight times as numerous as the Macaronesian species. The purely Canarian species would, in accordance with the theory of Dr. Willis, be the most liable to extinction, and this finds support in the behaviour of the *Staticeae* of the subsection *Nobiles* as described by Dr. Stapf ('Annals of Botany', xx, xxii). They are very local and 'there is a considerable risk of their total disappearance'. We should not look for the same with the Macaronesian species. But they must go the way of all plants, and one of them (*Clethra arborea*) has not been found since 1828.

In the more recent Flora of the islands by Pitard and Proust (1908, p. 77) we have important light thrown on the origin of new species in the Canaries. If they had not been following the practice long in vogue among systematists in dealing with insular floras, a practice well illustrated in the quotation from Hooker (already given) when he was discussing the origin of the Canarian endemic species thirty years before, one might have credited the authors with anticipating Dr. Willis in the matter of the Age and Area theory. But, as held by the writer, the real significance of this theory lies in its return to a pre-Darwinian position respecting plant distribution. Dr. Willis has here rendered the greatest service to botanical geography by demonstrating the importance of principles that had been almost forgotten in the efforts to apply the great theory of Darwin to the central problems of the plant world.

Viewed from the standpoint of Age there were for Pitard and Proust two types of endemic species in the Canaries. There were in the first place those found generally distributed over the group, very ancient forms that once existed in the neighbouring continent, but now survive only in the Macaronesian region. (These are the Macaronesian endemics before recognized.) Then there were the much more numerous recent forms, mostly localized in single islands and derived, just as Hooker held, from parent forms already in the archipelago. These are the purely Canarian species, and the authors of this Flora make some very suggestive remarks on Nature's mechanism in their production.

They take the case of the representatives of *Micromeria*, a cosmopolitan

Labiatae genus. Of the 20 Canarian species 15 are confined to one island, 3 to two islands, 1 to three islands, and the last, *M. varia*, a highly variable species, abounds all over the group, occurring also in Madeira. They assume that *M. varia* has probable descendants in species restricted to single islands and they extend this position to many other genera. We could not admit (they argue) in the cases of numerous other genera, *Statice*, *Senecio*, *Sonchus*, &c., where this occurs, that the species originally existed in all the islands and died out in all but one. Evidently they hold with Hooker that the single-island species have arisen as adaptations to the particular conditions of individual islands.

We have here spontaneous testimony that the rôle of the polymorphous or highly variable species in the development of new forms, a rôle which is so conspicuous in the later floral history of the islands of the tropical Pacific, is equally well illustrated in the Canaries. The principle is exemplified in its simplest shape in a compact genus or subgenus ranging over an archipelago and holding a score or so of species. Here a solitary highly variable species ranging over all the islands becomes the parent of several localized species that are often confined to single islands. But it is as true of a section of a genus characteristic of one group of islands as it is of a large genus distributed over many archipelagos and holding, as in the case of some genera of the Pacific, an ocean in its sway. It is as true of a subgenus confined to a small continental area as it is of a wide-ranging genus that covers a continent. It is often beautifully illustrated in the behaviour of a single species as a parent of numerous local races and varieties. It was the part taken by the polymorphous species in the Pacific, differentiating in every group and even in the individual islands of a group, that first led the writer to view the world of Nature as in the main a differentiating world. It represented for him in miniature a fundamental principle of distribution. Here on a small scale he recognized the process of the differentiation of the primitive generalized types that once ranged the globe.

The subject was first worked out by the writer in his volume on plant dispersal in the Pacific (1906), and it is there shown how numerous botanists, however much they might differ in other points, were at one in recognizing the play of the polymorphous or highly variable species in that region. The principle is either illustrated or implied in the case of one genus or another in the writings of most of the botanists who were interested in the floras of the Pacific Islands during the latter half of the last century and in the beginning of this, of Bentham, Burkill, Cheeseman, Drake del Castillo, Gray, Hemsley, Hillebrand, Reinecke, Rendle, Seemann, and others. Now we find it recognized in the Atlantic Islands, directly in the pages of Pitard and Proust, and by implication by Hooker for the Canaries in his reference to the 'generally accepted views' respecting the origin of localized endemic species in that group.

But the most significant fact in this connexion is that when Dr. Willis first promulgated his theory of Age and Area ('Ann. Roy. Bot. Gard. Peradenya', May 1907) his preliminary statement of it took this form. When he came to elaborate it he gave it the name of its most striking implication, Age going with Area. He arrived independently at the same conclusion respecting the plant-stocking of Ceylon and the adjacent mainland that botanists a generation ago had framed in the case of genera of the Pacific Islands. We have been apt to forget this preliminary statement of his position; but it is the essence of his theory. His views on the local origin of endemic species were first presented in this form:

'The general principle on which India and Ceylon have been peopled with the many species which they contain would seem to be that one very common species has spread widely, and so to speak shed local endemic species at different points, or else that one species has spread, changing at almost every point into a local endemic species, which has again changed on reaching new localities.' Examples are given of *Clematis* and *Anemone* in the Himalayas (we are told that many more cases could have been cited), where we have associated with a single species ranging throughout the region various allied species confined to particular localities. When Dr. Willis asked how these cases could be explained 'but on the parent and child theory' he put a question which many of us have asked.

But this principle is not merely insular. It is, as we have seen in the case of the Indian mainland, also continental. Whilst the highly variable species may cover much of a continent, its derivative species are restricted to small areas. Few better examples could be given than those of different species of *Geranium* over great areas of the Eurasian steppes, in the highlands of Mexico and Central America, over the length and breadth of the Andine region, and in the high mountains of the breadth of tropical Africa. The rôle of the wide-ranging polymorphous species, as a parent of localized endemic forms, has been as effective in the great mountainous regions of the continents as it has been over the archipelagos that dot the oceans. (As concerns *Geranium* the subject is lucidly treated by Knuth in his monograph on the Geraniaceae, 1912, one of the Pflanzenreich Series. pp. 79, 83, 85, 175, 185, 196, 202.)

It may be remarked in conclusion that of the several eminent botanists cited above not one could have been cognizant of any such theory as that of Dr. Willis. They have been named here in connexion with the important part played by the highly variable wide-ranging species in distribution, and it is on the behaviour of these polymorphous species that Dr. Willis based the preliminary statement of his views on the local origin of endemic species. His principle was often implied in the practice of these systematists, though not as an integral principle, but as part of a code that was none the less valuable because it was often unwritten. In theory a few

were Darwinian evolutionists, but many were not. The last, subordinating their theory to their practice, were, as I am willing to believe, 'differentiationists' at heart, holding, like their predecessors, ideas of divergence or differentiation of types, but ideas admittedly crude, and such as Hooker held in the shape of 'Centrifugal Variation' through much of his career. In their theory, as I take it, they followed Nature on her broadest lines, and they did their best to put it into their practice. This is the position to which the Age and Area theory will bring us back, a position that has been for a long while greatly obscured by the clouds of dust that have been raised in the Darwinian controversies. It is certain that this theory will be involved in a general differentiation hypothesis of some kind. Whether that position will be provisional or permanent, time will show. At all events the two views of Differentiation and of Age and Area seem to be inextricably bound together now.

List of the Macaronesian Species of Flowering Plants in the Canary Islands, being those also found in other Groups of the Macaronesian Floral Region (Azores, Madeiras, Cape Verdes), but not known outside it.

The letters after the names signify as follows: A = Azores; M = Madeira; V = Cape Verdes.

The arrangement adopted by Christ has been followed.

Ranunculus cortusaeifolius. M.	Sempervivum (Aichryson) radices-
Dichroanthus mutabilis. M	cens. M.
Lobularia intermedia. V.	Bencomia caudata. M.
Fumaria montana. V.	Cytisus stenopetalus. V.
Laurus canariensis. A, M.	Jasminum odoratissimum (barre-
Phoebe barbusana. M.	lieri). M.
Persea indica. A, M.	Picconia excelsa. A, M.
Oreodaphne foetens. M.	Heberdenia excelsa. M.
Hypericum (Androsacum) grandi-	Clethra arborea. M. ¹
florum. M.	Echium stenosphon. V.
„ glandulosum. M.	„ candidans. M.
„ floribundum. M.	Campylanthus salsoloides. V.
Visnea mocanera. M.	Lavandula pinnata. M.
Geranium anemoneifolium. M.	Microrneria varia. M.
Ilex canariensis. A (?), M.	Cedronella canariensis. M.
Rhamnus glandulosa. A (?), M.	Leucophaea massoniana. M.
Euphorbia mellifera. A (?), M (?).	Teucrium (Poliodendron) hetero-
Bupleurum salicifolium. M.	phyllum. M.

¹ Not found in the Canaries since 1828.

Globularia salicina. M.	Urtica morifolia. M.
Statice pectinata. V.	Myrica faya. A, M.
Phyllis nobla. M.	Dracunculus canariensis. M.
Viburnum sp. A, M. ¹	Tamus edulis. M.
Carlina salicifolia. M.	Ruscus (Semele) androgynus. M.
Andryala cheiranthifolia. M.	Smilax canariensis. A, M.
Sonchus pinnatus. M.	Asparagus scoparius. M, V.
Beta procumbens. V.	Dracaena draco. M, V.
Rumex maderensis. M.	

Note on the List of Macaronesian Species.

We are far from having done with the matter when we divide the endemic species of this group into those of the floral region (Macaronesian) and those peculiarly Canarian. Almost all the great problems in the plant-story of the region are involved in the list just given. They require a lengthy general discussion, and that being here impracticable we must be content with a bare list. Few of such lists are, however, available; and although this one cannot claim to be free from error, for botanists have made the Canarian one of the most difficult of insular floras, the writer believes that it will give a reliable preliminary notion of the general facies of a very interesting little gathering of plants. The short list bristles with implications, although for several reasons, one of which is given below, we have always to look outside the list to push them home.

Here we must limit the discussion to two of many curious points that arise as soon as we take the list in our hands. A genus originally represented by one or two species ranging over the floral region and by several species restricted to individual groups would ultimately, as differentiation proceeded, be only represented by species confined to single groups. For this reason it will be apparent that some of the most interesting genera of the Canarian flora will be absent from the list of Macaronesian species, their original wide-ranging species having disappeared in the differentiating process. For instance, of the four American genera, *Bowlesia*, *Bystropogon*, *Cedronella*, and *Clethra*, the third is the only one that strictly speaking ought to figure in the list. It is true that *Clethra arborea* is included, but it has not been found in the Canaries since 1828.

Another matter to be here noticed is the inclusion of the Cape Verde group in the Macaronesian region. For the best part of a century this has been a moot point, and it still remains so. In a physical sense these islands have been far more 'africanized' than those of the northern groups.

¹ There are indications that the Macaronesian forms of *Viburnum* are more allied to each other than to any continental plant, such as *V. tinus*.

The great climatic revolution that overwhelmed Northern Africa destroyed the old Tertiary continental forests of this archipelago. The only connexions with the northern groups that we should look for here would belong to what Christ terms 'xerothermic' types that are usually North African and Mediterranean in their range. This is pretty much what we find; but we cannot exclude these islands from the floral region. They must have belonged to it in the past, and they belong to it now, but in a limited sense. They come into it on a lower grade, and must be correlated with the lower or African zone of the Canaries and Madeira, both in a climatic and in a floristic sense. But the connexion with the northern groups, as Christ points out, is not merely a matter of identity of species. It is also concerned with affinity. There are a score or so of species nearly related to Canarian and Madeiran plants and of similar 'xerothermic' types (Hooker in his Lecture, p. 16, takes a similar view).

In framing the above list the works before named of Pitard and Proust and of Christ have been mainly used. But other sources of information have been consulted, such as Schmidt's 'Cap Verdische Flora', 1852; Coutinho's 'Catalogus Herbarii Gorgonei', 1914-15; Watson in Godman's 'Azores', 1870; Trelease on the Azores in the 8th Report of the Missouri Botanical Garden, 1897; and Lowe's 'Manual of the Flora of Madeira', 1857.

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Endemism in the Bahama Flora.¹

BY

NORMAN TAYLOR.²

With one Map in the Text.

SOMEWHAT over fourteen per cent of the wild flora of the Bahama Islands is confined to that archipelago. Among 894 native species scattered through the islands, 132 flowering plants are endemics, while of the balance, about 100 species are derivatives of cultivation or otherwise introduced.

While the rest of this paper will deal with the endemic flowering plants, the following is inserted to complete the known record of endemism in the archipelago:

	<i>Endemic Species.</i>
Spermatophyta	132
Pteridophyta	1
Bryophyta	1
Thallophyta:	
Fungi	18
Lichens	19
Algae (including Diatomaceae)	14
Myxomycetes	0
	<hr/> 185

The distribution of these endemic flowering plants and their near relatives appears to throw some light upon the floristic composition of the Bahama flora and upon that of adjacent regions. Before beginning a general or specific discussion of these endemics it may not be inappropriate to record certain facts regarding them that do not coincide with the 'Age and Area' hypothesis of Dr. J. C. Willis.

The substance of that theory demands that the antiquity of endemics should be measured by the amount of area they have covered. Very rare endemics would be quite 'new' while widely dispersed ones presumably more ancient. In a paper on 'Endemism in the Flora of the Vicinity of New York' it was suggested that at least in that region the youth or

¹ This study has been made possible by the recent publication of the very complete Bahama Flora by Nathaniel Lord Britton and Charles Frederick Millsbaugh. To both authors I am under pleasant obligations for help in the preparation of this account of the endemics. To Dr. Britton especially my acknowledgements are due for his interest in this study and his helpful suggestions during its progress.

² Contributions from the Brooklyn Botanic Garden, No. 25.

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antiquity of endemics could not be measured by their dispersal. For in some cases, particularly among the relicts of the pine-barrens, unquestioned antiquity goes with rather restricted distribution.

In the Bahama flora, necessarily a recent one, due to the geological youth of the islands, a somewhat detailed study of the endemics as contrasted with the non-endemic flora shows that these two floral elements are not widely different as to their dispersal.

	<i>Endemics.</i>	<i>Non-endemics.</i>
Found only on one island	29.5 %	20.3 %
Found only on two islands	14.5	13.7
Found only on three islands	13.6	9.3
Found on many islands	42.4	56.7

The essential similarity of these percentages warrants the statement that the dispersal of endemics in the Bahamas is not very different from that of the general flora of the archipelago. It may not be shooting beyond the mark to suggest that the dispersal, or, as Dr. Willis prefers to call it, the commonness or rarity of endemics, may be dictated by forces that play as well upon the endemic as upon the non-endemic elements of the Bahama flora.

Of those endemics confined to a single island, which constitute 29.5 per cent. of the endemic flora, and which might perhaps be considered the rarest, Dr. Britton has written the following for this paper :

'In almost all cases of which record was made by the collectors of endemics inhabiting, as far as known, only one island, the plants grow in large or considerable quantities. A few species are known from but a single specimen, or few, but wider search might reveal them in quantity.'

Both from the record of their distribution, and from the observations of those most competent to make them, it is thus apparent that the age of endemics in the Bahamas cannot be measured either by their dispersal or by their frequency.

The geological youth of the islands is reflected in the lack of endemic genera, only *Neobraccia*, a shrub of the Apocynaceae, being peculiar to the region. If, as most students of distribution agree, endemic genera are to be considered as badges of antiquity, and they have been so interpreted in St. Helena, Galapagos, Hawaii, and hosts of isolated islands, then the lack of them in the Bahamas should brand that flora with the stigma of youth, if the geologists had not already compelled us to do so. Such a recent flora, scattered over a rather restless archipelago, so far as subsidence and emergence is concerned, ought to show among its endemics a goodly proportion of herbaceous species. For it has also been shown, for at least some regions, that endemic species in non-endemic genera are mostly herbs, which from the brevity of their life-cycle are assumed to have greater opportunity to become developed than woody plants. As the Bahamas

are almost painfully new and have but one endemic genus, the rest of the 132 endemics should be overwhelmingly herbaceous. How far short they come of being so is shown below.

Percentages of Woody and Herbaceous Species in the Non-endemic and Endemic Elements of the Bahama Flora.

	Woody.	Herbaceous.	Parasites.
Endemic species	57.5	39.3	2.2
Non-endemic species	39.5	59.3	1.2

The endemic element of the Bahama flora is thus seen neither to fit into the 'Age and Area' theory of Willis, nor to accord with the theories of Sennott and Bailey. They have argued that endemism is a criterion of antiquity, particularly where that endemism is generic, when it is sure to be represented mostly by woody species. With only a single endemic genus and on notoriously youthful islands, 57.5 per cent. of the Bahama endemics are woody! In other words, during the time that these endemic species have been developing, considerably more woody plants have arisen than herbs, notwithstanding that in the total non-endemic flora, from which they sprang, the above percentages are reversed.

Recording the failure of Bahama endemics to support the contention as to 'Age and Area' or that of 'endemism as a criterion of antiquity', apparently puts upon us the burden of accounting for the unquestioned facts of their distribution in some other way. The islands have been so thoroughly explored by the authors of 'The Bahama Flora' and their associates that what follows regarding the dispersal of the endemic plants of the archipelago may be accepted with greater certainty than is usually possible in such cases.

ORIGIN AND DISTRIBUTION OF BAHAMA ENDEMICIS.

(a) *The Physical Features of the Islands and their Bearing upon the Flora.*

The dispersal of the wild plants over the archipelago has first of all been affected by the structure of the islands. As the accompanying map (p. 531) shows, there are really three groups of islands: (a) those that outcrop from the Little Bahama Bank, the larger of which are the islands of Great Bahama and Abaco; (b) those that outcrop from the Great Bahama Bank, notably New Providence, Eleuthera, Cat, Great Exuma, Long, and Andros islands; (c) a group of scattered and isolated islands, the chief of which are Watlings, Crooked, Acklin, Mariguana, Inagua, Caicos, and Turks islands. The outstanding fact about these three groups of islands is that those on the Little Bahama Bank have several times been connected among themselves, for the water on the bank is scarcely 20 feet deep, and evidence of

subsidence and emergence is unquestioned. Separating this group from those of the Great Bahama Bank, however, there is a passage of over five thousand feet depth. All the islands on the Great Bahama Bank are only just above the surface, the bank itself having scarcely 20 feet of water over it, so that they have been connected among themselves at some period of their history. All of the islands scattered to the southward of the Great Bahama Bank (group *c*) are surrounded by such depths of water that from it and from one another they must have been separated no matter what the local fluctuations of level throughout the archipelago may have been. Evidence collected by Alexander Agassiz and more recently by the Geographical Society of Baltimore puts the extreme changes of level throughout the islands as not over 200 feet. The oceanic depths between the Little Bahama and Great Bahama Banks and scattered islands to the southward are so great that no dry land connexion between them could have been possible. The authors of 'The Bahama Flora' say in the introduction to it, 'There is no evidence that there ever was land connexion with either Florida, Cuba, or Hispaniola' [Haiti], a statement abundantly justified by ocean depths often in excess of twelve hundred feet and not infrequently of over ten thousand feet.

The amount of emergence and subsidence of the islands is pretty accurately known, especially from the study of the ocean holes on the banks. The depths of even the shallowest of these indicate that at some period in the past, and not so very long ago, the archipelago must have risen so far out of the water that the Bahamas must then have consisted of one large island to the north, the Little Bahama Bank of to-day, another and much larger island to the south, the Great Bahama Bank, and a group of isolated islands to the southward, never connected with either of these large land masses, nor with each other. The largest of the group is Inagua.

The present Bahamas consist of wind and sea borne material piled up at the time of this emergence, all the area within the dotted lines on the map having since been covered by the sea, except for the islands exposed at present, the 661 cays, and thousands of rocks, almost awash, that make navigation so perilous.

(b) *Origin of Endemics.*

With only a single endemic genus, all the rest of the endemic species are in genera that are found either on the Florida mainland, the larger West Indies to the southward, or in many cases from more remote regions.

Of the 76 genera in which all the Bahama endemics are found, except the endemic genus *Neobraccia*, 47, or 63 per cent., are genera mostly containing numerous species of rather wide distribution. Nineteen, or 25 per cent., are genera found only in the West Indies or adjacent South

America, while 7 per cent. of Bahama endemics are found in genera that so far as known are confined otherwise to the Florida mainland. There also appears to be about 5 per cent. of genera containing endemics which are confined to the Florida-Bahama-West Indies region, and if this study were more extensive geographically they would be considered endemic genera. They are all woody plants. These Floridian-Bahamian-West Indian genera, with the number of Bahamian endemic species, are as follows:

<i>Torrubia</i>	With 1 Bahama endemic
<i>Rhacoma</i>	" 1 " "
<i>Malache</i>	" 1 " "
<i>Ernodea</i>	" 4 " endemics

These 76 genera, together with those in which no endemic species are found, make up the present flora of the Bahamas. As we have shown above, the distribution of this flora, both endemic and non-endemic, does not differ greatly, nor does there appear to be any reason why there should be any fundamental difference in the forces that have controlled the distribution of these plants over the archipelago. There can be no essential age difference so far as occupancy of the islands by their flora is concerned, whether the genera happen to contain endemics or not.

The present distribution of the endemics presents certain features that demand further study in the field. Recorded below are the percentages found in the different regions of the Bahamas, followed by a brief discussion of what these figures appear to indicate. It should be borne in mind that Great Bahama Bank and Little Bahama Bank include all the islands now exposed on them. As we have shown, the individual islands in either of these groups must have once been connected with one another but not with those of the other group. Nor has there ever been any connexion of either group with the scattered and isolated islands to the south. The percentages are as follows:

Distribution of Bahama Endemics on Islands of the Little Bahama Bank, Great Bahama Bank, or on Isolated Islands.

1. On Little Bahama Bank only	5.3
2. On Great Bahama Bank only	22.7
3. On separate islands only	35.6
4. On Little and Great Bahama Banks only	11.4
5. On Little Bahama Bank, Great Bahama Bank, and separate islands	9.9
6. On Great Bahama Bank and separate islands only	15.1

These percentages show some interesting features of the dispersal of endemics. In the first place the largest land mass, the Great Bahama Bank, has only 22.7 per cent. of the total endemics that are peculiar to it and now confined to the islands that outcrop from it. The second largest land mass, represented by the islands on the Little Bahama Bank, has only 5.3 per cent. of the total endemic flora of the archipelago. In other words,

72 per cent. of Bahama endemics (the last four items in the tabulation) are at the present time so distributed that they are found in essentially the same form in several places that have never been connected, and often separated by tremendous depths of the ocean. This fact is still further emphasized by the 35.6 per cent. of the total endemics which are confined to the group of isolated islands. Thus nearly three-quarters of the endemics show, by their dispersal over these islands, that (a) they may be relicts, which is somewhat confuted by the small number of endemics found all over the archipelago, less than 10 per cent.; (b) parallel evolution might account for them, although there is no evidence for or against such a theory, except possibly the great general similarity of habitat on practically all the islands; or (c) certain of these islands may always have supported these and other species, and from which they spread and may still be spreading.

As to the method of dispersal, the West Indian hurricanes have, as the record for the last forty years indicates, passed through the Bahamas in a generally north-westerly direction, but except for local effects such as the case of the 'Hurricane Grass', *Fimbristylis spathacea*, their action is assumed rather than proven. Studies in the field as to frequency of occurrence on different islands, on windward and leeward sides of the same island, upon the air-buoyancy of fruits and seeds, their times of ripening and falling as contrasted with the hurricane season, and some other matters, are necessary in order to make definite statements about the response of the Bahama flora to these violent storms. They are so tremendous in their ability to suck up small objects into the vortex, and move with such regularity about 12 to 14 miles an hour, and in such definite paths, that it would be surprising if they have not left their mark upon plant distribution. What that may have been demands further field study.

The Gulf Stream washes all the western side of the archipelago, but, without field studies of the ability of the seeds to both float and retain germinating power, such as the coco-nut is known to do, we can only conjecture about the influence of this great current. This applies not only to seeds that float directly, but to those that may become fastened to logs or débris and thus be carried from island to island.

Birds unquestionably carry seeds throughout the archipelago, but we as yet know very little definitely about the amount of this, nor do any of these three agencies of dispersal explain certain features of the distribution of the endemics. Over one-third of all the endemics are confined to the islands to the southward. With the Gulf Stream and hurricanes both moving past these islands in a north-westerly direction and yet failing to carry to a single island or key of the Great Bahama or Little Bahama Banks one of these southerly endemics, it is clear that the distribution of Bahama plants cannot be ascribed too readily to these agencies. Fifteen per cent. of all endemics, however, are found both on the Great Bahama

Bank and on the isolated southerly islands, while nearly 10 per cent. are found throughout the archipelago. That some of these have been distributed by the hurricanes or by the Gulf Stream is more than likely. But a comparison of them shows that they are often in genera which are also represented by the southerly island endemics which have failed to be so distributed.

While a fairly good case could doubtless be made out for parallel evolution as an explanation of similar endemics on geologically and edaphically similar but unconnected islands, proof of it is so far lacking. As suggested by Dr. Britton, the endemics of the Bahamas may be due to extreme isolation on certain islands which are very specialized by having peculiarly rocky and sterile soil, by the violence of the regular trade-wind which depresses the vegetation, and by the periodic hurricanes of most destructive force. If these factors have had any bearing upon the distribution of Bahamian endemics they should be better illustrated on the island of Inagua (including Little Inagua) than on almost any other.

It is the largest land mass of any of those southerly islands that have always been isolated by the great depth of the sea. Upon it grow a greater proportion of endemic plants than on any other island in the archipelago—49, of which 13 are confined to it. Andros, which is thrice larger, has only 5 endemics that are restricted to it, Long Island 4, and most of the rest of the islands one or no endemic peculiar to them. Inagua is thus seen to be not only abnormally rich in endemics, which comprise 21.4 per cent. of its recorded flora as against 1.4 per cent. for the whole archipelago, but it also has a higher proportion of endemics peculiar to it than any other island. Such a combination of circumstances warrants some special study of what these plants are. The list of endemics confined to Inagua or Little Inagua follows, together with some notes upon them.

Endemics confined to Inagua or Little Inagua.	Number of Endemic Species in the same Genus, but in other Parts of the Archipelago.	Total Number of Species known from the Bahamas.
<i>Dichromena inaguensis</i>	0	2
<i>Agave Nashii</i>	7	8
<i>Eucalyptus inaguensis</i>	1	8
<i>Heliotropium Nashii</i>	4	10
<i>Lantana bahamifera</i>	1	6
<i>Nashia inaguensis</i>	0	1
<i>Gneltardia Taylori</i>	0	6
<i>Gneltardia Nashii</i>		
<i>Gneltardia inaguensis</i>		
<i>Ernodea Taylori</i>	1	6
<i>Ernodea Nashii</i>	2	8
<i>Borreria inaguensis</i>		
<i>Veronica obcordata</i>	3	5

The ten genera in which these thirteen endemics peculiar to Inagua are found are distributed otherwise in the following way:

Dichromena has only one other Bahamian species, *D. colorata*, which is widely distributed both throughout the archipelago, on the mainland, and in the larger West Indies. It is found on Inagua, and must be the parent or source of the endemic *D. inaguensis*.

Agave has eight native species in the Bahamas, all endemics, which are scattered over the archipelago thus :

A. inaguensis on Little Inagua and on Caicos.

A. bahamana on several islands, all on Great Bahama Bank.

A. Millspaughii, confined to Great Exuma.

A. cacosela, confined to New Providence.

A. acklinicola, confined to Acklin Island.

A. indagatorium, confined to Watlings Island.

A. Braceana, on Abaco, Great Bahama, and doubtfully on Andros.

Encyclia has eight species in the Bahamas, with only one other endemic, *E. bahamensis*, which is found nearly throughout the archipelago and also on Inagua. *E. diurna* and *E. plicata*, both widely distributed in the Bahamas and elsewhere, are also found on Inagua. It is difficult to avoid the conclusion that from these three *Encyclias*, known to grow on Inagua, the endemic *E. inaguensis* has been derived.

Heliotropium has ten species in the Bahamas, of which five are endemic.

Of these endemics only two others are known on Inagua, while three common Bahamian *Heliotropiums* are also found there.

Lantana has six species in the Bahamas, two of which are endemic.

L. demutata is an endemic confined to the islands on the Great Bahama Bank. On Inagua are recorded *L. Camara* and *L. involu-crata*, from the latter of which *L. balsamifera* may be safely assumed to have been derived, as it is a plant of wide distribution nearly throughout the West Indies.

Nashia has only a single species in the Bahamas, the endemic *N. inaguensis*, confined to that island. Two other species are known in the larger West Indies.

Guetlardia has six Bahamian species, with all its three endemics confined to Inagua. The other three species, all found on Inagua also, are of wide distribution both in the Bahamas, the Florida mainland, and in the West Indies.

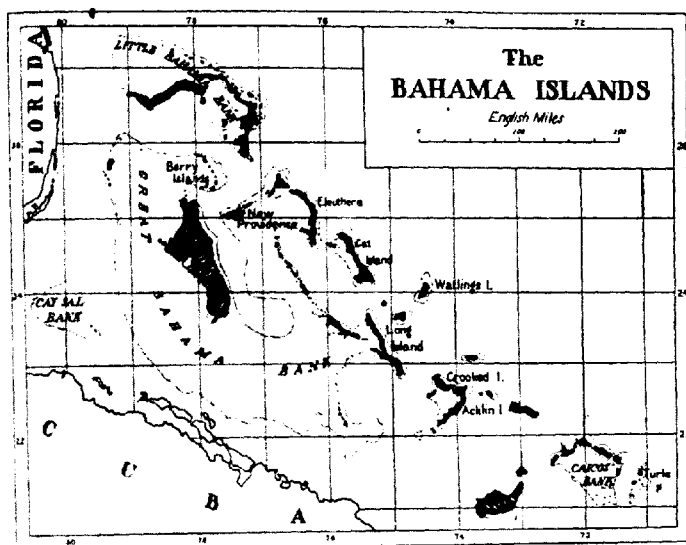
Ernodea has six Bahamian species, four of which are endemic. Two of these endemics are unknown on Inagua, but *E. littoralis*, a species of wide distribution, is common there. *E. Nashii* and *E. Taylori* must have been derived from it, for these comprise all the *Ernodeas* known on the island.

Borreria has eight species in the Bahamas, six of which are endemics. Of these endemics *B. savannarum* and *B. bahamensis* are found on Inagua as well as other islands, while the only widely distributed species found on the island is *B. laevis*.

Vernonia has five species in the Bahamas, four endemics among them.

No other *Vernonia* is known on Inagua but the widely distributed *V. bahamensis*, which is also endemic in the archipelago, and from which the endemic *V. obcordata* peculiar to Inagua must have been derived.

In all these ten genera, except *Agave* and *Nashia*, it is difficult to avoid the conclusion that endemics peculiar to Inagua have been derived from other and much more widely distributed species also known to grow there. *Agave*, with all its species endemic, and only one of which besides *A. Nashii* is also found on Inagua, is a peculiar case. In this genus



Note the dotted lines that mark the limits of the old land masses of the Bahamas, now represented by the Banks and the scattered islands.

we are confronted with a wealth of scattered progeny but a dearth of parents. The most widely distributed endemic is *A. bahamana*, but it is unknown from Inagua, and is so far recorded only from the islands on the Great Bahama Bank. In other words, we are faced with two endemic century plants on Inagua, and with *Nashia inaguensis*, none of which have any relatives now on the island from which they could have been derived.

There is, of course, always the chance that the progenitors of these endemic century plants and of *Nashia* have failed of survival, and that these endemics and others in the archipelago represent the end-series of a pre-existing flora, which the great specialization of habitat has produced. The sterility of the soil, limited rainfall (salt is recovered by evaporation of

sea-water in several islands), and the saline soil conditions in many others are reflected in a generally depressed vegetation with many adjustments to prevent transpiration. A field study of the origin and distribution of the flora of Inagua and some of the limiting factors would throw much light on this problem. The study of the records of 'The Bahama Flora' shows Inagua to be higher in endemic species than any other island in the archipelago. It is the largest land mass of any of the isolated islands, and there is in the interior of it a good-sized salt lake and savannah region. These combinations of special conditions, reflected in its flora as they are known to be, make it worth additional field study.

SUMMARY.

Endemism on it, and in fact throughout the archipelago, appears to be the response of vegetation to these peculiar conditions of soil, lack of rainfall for a region only 70 miles from the rain-forest of northern Cuba and Haiti, regular and rather strong trade winds, punctuated by violent hurricanes in the opposite direction, and by certain other factors. In Inagua, more than anywhere else in the archipelago, the sum of these factors has produced endemic species. As has been shown, the distribution and growth form of these endemic species does not differ materially from the non-endemic ones.

The comparatively short time in which this endemic flora must have been developed, its method of transportal from island to island or from the mainland, these and other matters regarding the distribution of the Bahama endemics need further field study. Such a study should include Inagua for reasons that need not be repeated here.

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Root Development in Barley and Wheat under Different Conditions of Growth.

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With four Figures in the Text.

IN 1918 our attention was drawn to the occurrence of white roots in wheat growing in the field. These white roots were easily distinguished from the ordinary type by their thickness, their waxy appearance, and by a lack of lateral branches. They were noticed on plants in early spring, and it was suggested to us that they form an extra food-supplying system for the wheat plants when they are beginning their summer growth. This phenomenon, however, has been observed at Rothamsted for many years in different series of experiments when barley is grown in water cultures. The white roots are found fairly abundantly on all the plants when they are taken out of their solutions at the conclusion of the experiments, which generally occurs before the barley is fully ripe. A typical root system under these circumstances shows a number of very long roots of medium thickness, which occupy the central part of the system and branch freely throughout almost the entire length. On the outer side of the root, springing from the tillers, and therefore of later growth, are usually a number of 'white roots', which are much thicker than the others and often reach a length of several inches without bearing any laterals. Some of the longest white roots bear a few short laterals near the crown. Together with these thick roots are thin, delicate ones which begin to give off laterals while quite small, and tend to branch well down towards the tip. 'White' roots have also been observed on wheat plants in Broadbalk in early spring, occurring earlier in the farm-yard manured plot than on the unmanured. The occurrence of these white roots in such widely different circumstances as those obtaining in water and in soil cultures led us to investigate their

appearance in plants at different periods of growth, and the experiments were extended to include the relations between root and shoot growth.

A considerable amount of information is available on the subject of the growth of the shoot and of the root of various crop plants, but there is not much literature dealing with the relation between shoot and root growth at various stages in the plant's history.

Experiments were carried out this year (1920) to investigate the root systems of plants growing in pots under controlled conditions and also of others growing under ordinary farm conditions in the field, and an attempt has been made to correlate shoot and root development. Wheat and barley were chosen for this investigation, both in the pot cultures and the field trials.

ROOT SYSTEM OF BARLEY.

Pot Cultures.

The early stages of development were obtained by sowing the grain in small glazed earthenware pots, 7 inches in height and $5\frac{1}{2}$ in diameter, holding $6\frac{1}{2}$ lb. of soil. The soil used throughout the experiment was rather heavy farm soil mixed with 10 per cent. of sand to lighten it. Half the pots were unmanured and half had 0.7 grm. superphosphate mixed in with the soil. In each manurial series one half of the pots were sown with wheat and the other half with barley at the rate of two grains per pot in each case. When the shoots were about 1 in. high, the weaker plant in each pot was destroyed by cutting off the shoot, since pulling up by the root would probably have disturbed the roots of the other plant. At the same time a series of cultures in large pots, height 14 in., diameter $6\frac{1}{2}$ in., each holding 23 lb. soil, were set up, $\frac{2}{3}$ of which were left unmanured and $\frac{1}{3}$ received $2\frac{1}{2}$ grm.¹ superphosphate per pot. Seeds were sown as before—wheat on Feb. 21, barley on March 5. Some weeks later, on April 6, sodium nitrate and potassium sulphate were applied as top-dressings to some of the pots, the final series of manuring being as shown in the following table:

Series	1	Unmanured.
"	2	" + sodium nitrate.
"	3	Superphosphate
"	4	" + sodium nitrate.
"	5	" + potassium sulphate.
Wheat sown Feb. 21, weight of grain 0.05–0.06 grm., top-dressed April 6.		
Barley " March 5, " " " " " " "		

The scheme adopted was periodically to examine the root growth of two plants with each type of manuring. The small pots were examined

¹ This is at the same rate as in the small pots.

weekly, the large ones fortnightly, but later the period was lengthened, as the supply of plants threatened to run short before maturity was reached. For convenience of working, wheat and barley were dealt with in alternate weeks.

As soon as the seedlings appeared above ground in the small pots, which was about ten days after sowing, two unmanured pots and two superphosphate pots were very carefully emptied. The plants were extricated without damaging the roots, and the latter were then washed in water with a camel's-hair brush. Sketches of the plants were made and full notes taken on the condition of root development. The following weeks this proceeding was repeated until all the small pots were emptied. These early plants were not weighed, as it was not at first realized what a large amount of root growth would be obtained under the given experimental conditions. By the time the small pots were finished the top-dressings had been applied to the large pots, and henceforward two pots from each manurial series were dealt with at a time, making ten plants in all each week. It was of course impossible to empty out these large pots without damaging the roots, and therefore a jet of water was used to wash away the soil without injury to the delicate roots. In the method adopted the pots were placed in a horizontal position on a stand of convenient height, with the mouth projecting over a large bath, and the soil was washed out of the pot by means of a small but fairly strong jet of water. Most of the soil was caught by the bath, thus preventing the troubles of drain stoppage, and if by chance a portion of the roots became detached it was more easily reclaimed than it would have been if the washing out were done directly over a drain. In using the jet of water great care had to be exercised to avoid dislodging the soil in the middle of the pot first, for if this happened the superimposed soil collapsed suddenly and in so doing tore away several of the roots. After removal from the pots the roots were carefully freed from adhering particles of soil with a camel's-hair brush, and were then disentangled and measured under water to prevent any shrinkage due to drying up. The length of the shoot and the number of tillers were noted and after drying the roots and shoots were weighed separately.

A well-developed root system of barley examined half-way through growth showed two distinct types of root: (a) long, much-branched roots, forming the bulk of the system, and (b) short, white, unbranched roots.

(a) *Branched roots.* Seven weeks after sowing the length and number of the *branched roots* varied considerably with the type of manuring, the highest number occurring with the superphosphate, and the lowest with the unmanured plants. In the more heavily manured plants laterals were well developed and tended to become concentrated in the upper 10-12 in. of the roots, while in the unmanured and the nitrate only plants the laterals were not so well developed and were much more scattered. As growth proceeded,

this concentration of the laterals in the upper part of the roots was noticeable in all the manurial series. In the early stages of growth the addition of superphosphate seemed to bring about considerable development of laterals, but in the unmanured and in the nitrate plants laterals were fewer, they were less branched, and apparently bore fewer root-hairs than those receiving superphosphate. In the unmanured plants this condition remained throughout the experiment, but in the plants receiving nitrate the laterals became very coarse and were much concentrated in the part nearest the grain, giving a crinoline effect. This effect was produced by the formation of a large number of extra long laterals along the few inches of root near the grain, rather than by the formation of numerous roots practically all at one point. The plants receiving superphosphate and nitrate also showed this crinoline effect very strongly. At later stages of growth the roots of the nitrate and of the superphosphate and nitrate-treated plants had become so densely matted together that it was impossible to decide whether the crinoline effect was still present or not. At maturity there was certainly no such effect observable in the nitrate only plants, for by this time the total amount of root system was very much smaller than it had been at earlier stages. In the superphosphate and nitrate plants, however, the crinoline effect was still observable at the last examination. The plants with superphosphate and potash also showed some crinoline effect, but this was not as marked as that of the nitrate plants. A noticeable feature in the potash series was the thickening and stiffening of the roots near the crown, where the laterals also were of a coarser type than elsewhere. This stiffening caused the roots to stand well away from the plant, and even when they were taken out of water the roots showed no tendency to collapse against each other, as they did in plants from all the other manurial series. As this did not occur in the plants receiving superphosphate only, it was presumably an effect due to the potash. Again this effect was not observed at maturity, when the root system was very much reduced in size.

(b) '*White*' roots. The plants were first examined eleven days after sowing, when the shoots were about half an inch above the soil. Seven or eight roots were present, the longest being about 5 in.; lateral branches had not made their appearance, but root-hairs were very plentiful, especially near the grain. Seven days later laterals had developed on all the roots, and as growth proceeded there was an increase in the number and length of the roots and a similar increase in the development of laterals. It was not until four weeks after sowing that the first '*white*' roots made their appearance. At this time the shoots of the unmanured plants were about 11 in. long with no tillers, the main roots being about 15-20 in. One plant examined showed a root about $\frac{1}{4}$ in. long coming from the base of the grain, of a much thicker nature than any of the others and of a dead white colour

with a waxy appearance. The superphosphate plants were well ahead of the unmanured; the shoots were longer and had commenced to tiller, and the root system was much stronger. Each plant showed one thick white root over 1½ in. in length. At this time the top-dressings of sodium nitrate and potassium sulphate were applied, and a fortnight later in all the manurial series the 'white' roots had become more numerous, and some had reached a length of several inches. In many cases they came off from the node just above the grain. In the unmanured and in the nitrate series the number and average length of the 'white' roots were very much the same, and they remained comparable for the next four weeks, i.e. until eleven weeks after sowing. With superphosphate, root growth was encouraged from a very early date, and as time went on the differences between these plants and the unmanured were accentuated, as is seen by comparison of the number of white roots at different periods in the following table (I).

TABLE I.
Barley (sown March 5).
Showing average number of white roots at different dates.

Age of plant.	Date of observation.	Unmanured.	NaNO ₃ .	Superphosphate.	Superphosphate + NaNO ₃ .	Superphosphate + K ₂ SO ₄ .
Weeks.						
7	April 20	3*	4	6	5	5
9	May 4	7	4	15	18	15
11	May 18	17	17	22	25	24
13	June 8	0	0	0	0	0

* Each number is the average of two plants.

At first the plants with superphosphate and nitrate gave lower numbers of white roots than did the superphosphate alone, but later the former drew ahead. With superphosphate and potash the numbers were much the same as with the superphosphate alone (see Table I). The length reached by the white roots did not seem to be affected by the type of manuring, but ranged up to about 9 in., according to the age of the roots.

These white roots do not retain their unbranched character throughout the life of the plant, but after some time they put out laterals and ultimately approximate to the general root system. When all the plants were eleven weeks old, a marked change was noticed in the formation of the root system. The thick white unbranched roots were no longer present, though a certain number of short roots occurred which were thin and showed incipient laterals. With the superphosphate and nitrate manuring a few thick white roots were still noticeable, all of which carried laterals well down their length, while with the superphosphate and potash a good number of long, thick, white roots with a number of laterals in the upper 4 in. were noticed. From this time onwards the 'white' roots entirely

disappeared, but a large number of thin roots bearing only incipient laterals were present until three weeks before harvest time. When the last plants were taken out, all the roots showed normal branching.

Considering the manurial series together, a feature common to them all is the steady rise in the numbers of the white unbranched roots for the period extending from the 7th to the 11th week after sowing and their disappearance after that time.

Relative growth of root and shoot in Barley. The relative growth of the root and shoot, as measured by the amount of dry matter produced, shows an interesting correlation with the morphological development of the root at different periods of growth (Table II and Fig. 1).

For the first six or seven weeks growth was very slow, and the plant seemed to be laying the foundations for future development. The actual difference between the dry weight of root and shoot after seven weeks was not very great, but by this time the beneficial effect of the superphosphate which was added at the beginning was already marked. During the next fortnight a rapid increase in growth took place, and a still further increase occurred afterwards, the latter being more marked in the shoot. In some cases the shoots continued to increase in dry weight till harvest time, though in others a slight fall was indicated towards the end. The roots, on the contrary, reached their maximum weight long before the harvest, the exact period varying with the manures applied. The unmanured roots, as would be expected, made less growth than any others: they increased in dry matter for about seventeen weeks and then gradually lost weight till harvest, seven weeks later. The biggest root growth was given by sodium nitrate, much the same result being obtained whether superphosphate was present or not. The general effect of manuring was to hasten the rise to a maximum, which was reached in most cases after fourteen weeks' growth, though in unmanured soil and in the presence of superphosphate and sodium nitrate together increase continued for seventeen weeks. From the maximum a fairly rapid and steady decrease in dry matter took place until at harvest time most of the roots, with all types of manuring, were of much the same weight and were reduced to what they had been after only ten or eleven weeks of growth.

This decrease in weight appears to be associated with an actual loss of root material due to decay or to migration into the aerial parts. At the time the roots were at their maximum they were strong and well developed, but afterwards at each successive washing out it was obvious that depreciation was taking place, till by harvest time the roots were very poor and feeble.¹

¹ The superphosphate and sodium nitrate root seemed to be an exception to this, as the last plant observed had a very massive and heavy root. Unfortunately only one plant was available at this date, but it is quite possible that this may have been an exceptional one, as those observed three

TABLE II.
Dry weights of individual Barley Plants.
Seven March 5, 1920.

Date.	Unmeasured.			Sodium nitrate.			Superphosphate.			Superphosphate + Sodium nitrate.			Superphosphate + Potassium sulphate.		
	Shoot.	Root.	Total.	Shoot.	Root.	Total.	Shoot.	Root.	Total.	Shoot.	Root.	Total.	Shoot.	Root.	Total.
April 20	0.20	0.11	0.31	0.20	0.13	0.33	0.42	0.25	0.67	0.22	0.13	0.35	0.36	0.18	0.54
	0.14	0.09	0.23	0.21	0.11	0.32	0.42	0.28	0.70	0.33	0.25	0.58	0.41	0.24	0.65
May 4	0.49	0.34	0.83	0.36	0.22	0.58	1.41	0.90	2.31	2.01	1.20	3.21	2.07	1.48	3.55
	0.58	0.39	0.97	0.61	0.42	1.03	2.35	1.28	3.63	1.84	1.07	2.91	1.71	0.81	2.52
May 18	1.62	1.54	3.16	1.78	1.21	2.99	4.11	3.52	7.63	5.65	3.96	9.61	5.75	4.13	9.88
	2.25	1.66	3.91	2.63	2.06	4.69	2.06	1.27	3.33	6.24	3.69	9.93	5.45	3.97	9.42
June 8	5.29	1.98	7.27	11.10	5.93	17.03	10.90	4.84	15.74	13.09	4.71	17.80	14.61	4.84	19.45
	9.53	3.21	12.74	13.99	6.67	20.66	12.98	5.66	18.64	11.56	4.66	16.21	14.74	4.90	19.64
June 19	12.61	3.15	15.66	18.50	5.15	23.65	18.22	3.33	21.55	22.09	6.51	28.61	21.00	4.73	25.73
	14.32	2.87	17.19	18.87	5.74	24.61	17.22	4.23	21.45	22.17	5.35	27.52	20.30	4.61	24.91
July 27	21.23	1.95	23.18	20.97	3.65	24.62	18.62	2.31	20.93	26.76	4.16	30.92	21.79	1.79	23.58
	13.65	1.19	14.84	28.45	4.09	32.54	21.26	3.00	24.26	26.78	4.41	31.19	22.49	1.55	24.04
August 17	17.67	1.47	19.14	22.21	1.84	24.05	20.41	1.58	21.99	27.85	5.44	33.29	21.72	1.34	23.06

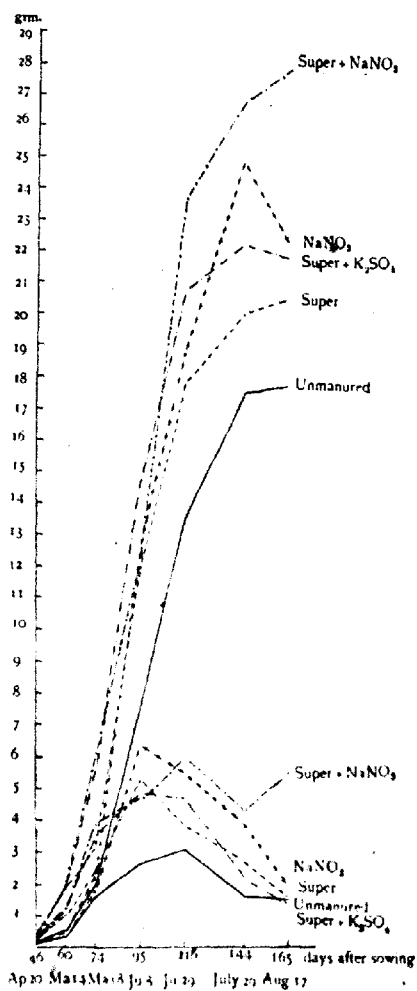


FIG. 1. *Barley*, plants grown in pots. Dry weights of shoots, upper curves; dry weights of roots, lower curves. (Mean of two plants in each case.)

It may be suggested that part of the loss may have been due to the roots becoming more and more brittle and breaking off at the ends in the process of washing. If, however, this had been the case to any marked degree traces of the broken roots would have been observed during washing, as this was conducted with the utmost care and watchfulness. Corroboration may be obtained from two sources.

(a) When barley plants are observed in the field the root system at harvest time seems small in comparison with what it is at earlier stages of growth.

(b) In water cultures in which peas were grown, dried and weighed at regular intervals during growth, a definite fall in the weight of the root set in some time before the plants had completed their growth. The fall in this case must necessarily have been real, and due to decay of plant tissues, as under the particular experimental conditions it was possible to recover the whole of the root without danger of loss.

In some cases it appeared that the maximum root development was reached at about the time that the ears were ready to emerge from their sheaths, i.e. at the time that pollination and fertilization of the ovule were about to take place. With superphosphate alone and with nitrate alone, however, this maximum was reached somewhat earlier, so that apparently root growth culminated with the final stage of preparation by the plant for grain formation. In other words, during the period of purely vegetative growth the plant needs large supplies of nitrogen and ash constituents to aid in building up a strong shoot in readiness for grain formation, and the root steadily increases in order to be able adequately to cope with this demand. During the reproductive phase, on the other hand, vegetative development is reduced to a minimum, and the whole of the plant's energy is diverted towards the grain. Although nitrogen and ash constituents are now just as essential as before, the area of supply is increased, as migration of these substances from the straw into the grain goes on from the outset.¹ This reduces the strain on the root, and as such a large absorbing area is no longer required it appears that the excess provision may be got rid of by a steady process of decay. Analyses indicate that by the time desiccation of the grain sets in, about three weeks before harvest, the whole of the nitrogen and ash required is already in the plant, so that the work of the root, other than as a water-absorbing organ, is practically complete.

weeks earlier showed a similar decrease in weight to all the other types, and if this decrease had continued its normal course to the end the superphosphate and nitrate plants would have fallen into line with all the others.

¹ Brenchley, W. E. (1912): The Development of the Grain of Barley. *Ann. Bot.*, xxvi, pp. 914-19.

542 *Brenchley and Jackson.—Root Development in Barley and*

Shoot/root ratio. The relations between the root and shoot at different periods are well brought out by a comparison of the shoot/root ratios.

TABLE III.
Shoot/Root Ratios. Barley.

Date.	Unmanured.	Sodium nitrate.	Super-phosphate.	Super-phosphate + Sodium nitrate.	Super-phosphate + Potassium sulphate.
April 20	1.75	1.86	1.69	1.70	1.98
	1.58	1.92	1.52	1.32	1.75
May 4	1.45	1.59	1.55	1.68	1.40
	1.52	1.45	1.84	1.72	2.11
May 18	1.06	1.18	1.17	1.43	1.39
	1.36	1.28	1.62	1.69	1.37
June 8	2.67	1.87	2.25	2.78	3.02
	2.97	2.10	2.30	2.48	3.01
June 29	3.97	3.59	5.47	3.38	4.43
	4.99	3.29	4.07	4.70	4.41
July 27	10.89	5.75	8.07	6.43	7.81
	11.48	6.97	7.10	6.08	14.49
August 17	12.02	12.05	12.95	5.12	16.23

For about a month after rapid growth set in both root and shoot grew at much the same rate. If anything, root growth was the stronger, as the shoot/root ratio had a tendency to fall throughout the period. From this time on the shoot grew far more rapidly than the root, and the ratio immediately increased, to be followed by a still further rise after the weight of the root had reached its maximum and had begun to fall. With superphosphate and nitrate, either alone or combined, the disproportion between shoot and root was less marked than with no manure or mixed minerals (superphosphate and potassium sulphate). (See Table III and Fig. 2.)

The change in the relative ratio of growth of root and shoot is closely associated with the change in the morphological structure of the roots. The 'white' roots, described earlier in the paper, were markedly in evidence for the first eleven weeks of growth, during which the shoot and root were growing at much the same rate, and the shoot/root ratio remained practically constant or showed a slight fall. With the increase in the rate of shoot growth and the consequent rise in the shoot/root ratio came the disappearance of the 'white' roots—the change being quite sharply marked in every case. This change occurred at the same time with all the systems of manuring, although the actual amounts of previous growth were so different. Possibly this was associated with weather conditions. Prior to this time, about May 18, the mean* maximum temperature did not rise above 60° F., nor the mean minimum above 45° F. After this the mean maximum did not fall below 60° F., and the mean minimum was usually

over 45° F. Also an extra amount of sunshine occurred just at this time, 82.5 hours for the week (the maximum for the whole season), and this may have helped to cause such a striking and sharply marked change.

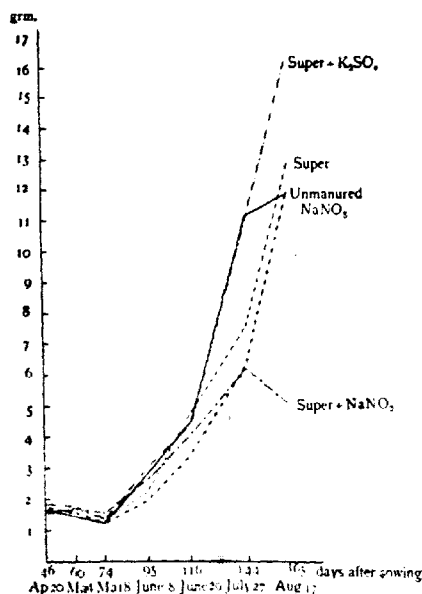


FIG. 2. Barley, shoot/root ratio.

Field Trials.

Methods used by earlier workers. Various methods of root investigation have been devised at different times, the most straightforward being to wash out the roots by means of a jet of water under pressure. Hays,¹ working at Minnesota in 1889, used this method and was able to obtain useful information on the development of the roots of maize plants. A few years later,² 1893, he used a more complicated method by which he was able to extricate the plant with its roots in almost the exact position in which they grew. This was done by means of a frame made of 1 in. gas-pipe, which was sunk into a hole just large enough to take it. The frame

¹ Hays, W. M.: Corn, its Habits of Root Growth . . . Suckers. Agr. Expt. Sta., Univ. Minnesota, Bull. 8, pp. 52-53, 1889.

² Ibid.: A Device for Illustrating the Root Growth of Plants. North Dakota Sta., Bull. 10, pp. 47-9, Pl. 1, 1893.

was filled with sifted soil and at each 2 or 3 in. a square of 2 in. wire netting was placed and each square wired to the corner parts of the frame. Two or three seeds were sown in each frame, and the seedlings when well established reduced to one. When a plant was almost mature, the soil was washed out of the frame with a spray; in this way the roots were left spread out in the different layers of netting. In 1892 another American worker,¹ F. H. King, made comprehensive studies of maize roots growing in the field. A trench 2 ft. wide was dug, leaving a prism of soil 1 ft. thick and extending at right angles across two rows of corn, so that a hill of corn stood at each end of the prism. The trench was deepened until all roots had been passed. The prism of soil was then fitted with a cage made of galvanized iron and wire netting, and when this was in place sharpened wires were pushed through the soil in parallel lines along the meshes of the netting. The wires reached right through the soil prism and were fastened at each end of the netting. The loose surface soil was taken off and replaced by a block of plaster of Paris; then the soil was removed from the cage by a force-pump with a stream of water $\frac{1}{8}$ in. in diameter. This method has been adopted by several investigators; Goff² in 1897 used it for examining the root systems of raspberry, strawberry, grape, and apple, while Ten Eyck³ in 1900 and Shepperd⁴ in 1905 employed the method for various field crops. A more recent investigation was carried out by Maschhaupt in 1915.⁵ Having selected a shaft of soil of sufficient breadth and depth to include the root systems of the plants under investigation, he cut away the soil from one side. A board studded with needles of sufficient length to penetrate the breadth of the shaft was then driven in a vertical position against the exposed side, the needles entering horizontally and so keeping the roots in their original position. The shaft was then cut loose on the opposite side by driving down to the desired depth a piece of sheet iron, and the soil was carefully washed away from the roots.

Rothamsted methods. The field trials which were started here were intended for comparison with the pot cultures, and five plots were set out for wheat and five for barley. Their arrangement is illustrated in the accompanying table, the manurial system being exactly the same as that followed in the pot cultures. The wheat and barley were sown on Feb. 25, and twelve days later several plants were dug up, brought down

¹ King, F. H.: *Natural Distribution of Roots in Field Soil*. Wisconsin Sta. Report, 1892 and 1893.

² Goff, E. S.: *A Study of the Roots of Certain Perennial Plants*. Wisconsin Sta. Report, 1897, pp. 286-98.

³ Ten Eyck, A. M.: *A Study of the Root Systems of Cultivated Plants grown as Farm Crops*. North Dakota Sta. Bull. 43, 1900, pp. 532-50.

⁴ Shepperd, J. H.: *Root Systems of Field Crops*. Ibid. 64, 1905.

⁵ Maschhaupt, J. G.: *Root Systems of Agricultural Plants*. Verslag Landbouwk. Onderzoek. Rijkslandbouwproefstat. (Netherlands), No. 16 (1915), pp. 76-89.

to the laboratory, washed in water with camel's-hair brushes and sketched. This was repeated twice at intervals of about ten days, by which time the plants were too large to be handled in this way. The crop of both wheat and barley was very poor, as not only were bird attacks very severe, but the ground was almost covered with weeds, chiefly *Papaver rhoeas*, *Matri-caria inodora*, and *Sonchus arvensis*. The barley crop was better than the wheat, and when the barley was almost ripe a trench was dug across the

<i>Wheat.</i>		<i>Barley.</i>
1	Superphosphate only	6
2	Unmanured	7
3	Superphosphate + NaNO_3	8
4	NaNO_3	9
5	Superphosphate + K_2SO_4	10

plot receiving superphosphate and the unmanured plot (6 and 7 in table). An attempt was then made to wash out the roots with the spray from an ordinary spraying machine. Very little root system had developed, probably owing to the extremely poor condition of the soil. It was therefore decided to examine the roots of some of the ordinary farm barley, and another trench was dug in one of the fields where there was a good crop of unripe barley. The root systems here were rather better than on the experimental plot, but it was exceedingly difficult to wash them out without breaking the roots, for the soil was very hard; about 2 in. at the surface were moderately friable, but below that came layers of very clayey soil which became a sticky mass when water was sprayed on to it. By examining the sides of the trench it was found that very few roots penetrated below the top 2 in. or 3 in. and none appeared below the 6 in. level. Another method for obtaining the roots was then tried. A small cluster of healthy plants was chosen and a 12 in. cube of soil dug out, with the selected plants in the middle of the cube. The solid block was transferred to a box which just held it and brought down to the laboratory, where the roots were washed out in exactly the same way as those in the pot cultures. Plants from ground which had received farm-yard manure and from ground manured with superphosphate were treated by this method, and the results described below.

Barley. Growth of Roots in the Field.

The field barley was first examined twelve days after sowing. At this time the shoots were not visible above ground, but each plant showed six or seven roots ranging from $\frac{1}{2}$ in. to 1 in. in length. Twenty-one days later, i.e. thirty-three days after sowing, the number of roots was much the same, 6-8, but the length had increased to about $4\frac{1}{2}$ in. and laterals were developing. The superphosphate plants showed a rather

better development of laterals than did the unmanured, but no other differences, either in size or general appearance, could be observed. The original intention was to examine the field roots periodically, but it was found impossible to do this and to carry on the root washing at the laboratory at the same time. Accordingly the trench method for examining the roots was not attempted until August, 168 days after sowing. At this time the barley was mature, and very little root system was found either in the unmanured or in the superphosphate plots. This may have been due to several causes; possibly the root system had been better earlier in the season, but the amount of root had decreased as the grain matured, a condition similar to that found in the pot cultures. The conditions of growth were as follows: The tilth was poor and the soil was in a very starved condition, no manure having been applied for years, and the clayey subsoil approached very near the surface. These conditions may easily have affected the roots adversely. Several of the larger roots were traced to their tips and most of their laterals were found to be perfect, but few were longer than $\frac{1}{2}$ in., nor were they branched. The majority of the roots were confined to the upper few inches of the soil, the roots running down at a slight angle to a depth of 3 in. or 4 in. When the poor root development was discovered the experimental plots were abandoned and the ordinary farm barley was examined as described above. This soil was in better condition than that on the experimental plots, and the barley was still in the green stage. About four plants grew together in the drill, giving the appearance of one tillered plant, whereas in reality very few of the plants showed any tillering. The root system was mostly confined to the top 2 in. of soil; some roots ran down obliquely into the soil, and a few went down vertically, but none of them were observed to reach below the top 6 in.

The plants washed out from the cubes of soil (see method above) had been manured with superphosphate. They showed 2-4 tillers per plant, and a fairly well developed root system. From each crown a number of short roots, $1\frac{1}{2}$ to $2\frac{1}{2}$ in. long, spread out in all directions, penetrating to a depth of 1 in., while a few roots were longer, much branched, and ran down obliquely to about 8 in. The greater part of the root system, however, was again in the top 2 in. Each plant showed one or two roots which had rotted back to a point above 5 in., which would correspond to a depth of 3 to 4 in. in the soil; probably the root here entered a bad stratum, which could not be penetrated. Some rather thicker and less-branched roots were observed coming from the node, while the finer, more branched roots came from the grain. The plants grown with farm-yard manure were much better than any of the previous field specimens—a typical plant having about seven tillers and many long roots with abundant laterals concentrated in the upper 5 in. of the root. There were

no roots here comparable to those found in the superphosphate plants, where the ends had become rotten. This may be due to the better tilth obtained in a soil treated with farm-yard manure. Several of the roots penetrated to a depth of 9 in., and again it was observed that the roots coming off from the node were rather thicker and less branched than those coming from the grain.

ROOT SYSTEM OF WHEAT.

Pot Cultures.

In wheat, as in barley, a strong root system developed, the types being more or less similar except with regard to the behaviour of the white roots. Nine days after sowing, when the shoots were about $\frac{1}{2}$ in. long, the roots of both the unmanured and the superphosphate plants were very similar. A typical plant possessed 3 roots up to 4 in. long, with abundant root-hairs. Five weeks after sowing the unmanured plants were found with 4, and the superphosphate with 5 roots, the longest of which in both cases reached 21 in. These numbers are rather lower than those obtained in the barley plants, where 7 or 8 roots were developed in seedlings 5 weeks old, but the maximum length of root was the same for both wheat and barley. Lateral development was consistently stronger in all the manured plants than in the unmanured, and in all series the laterals showed a tendency to concentration in the upper 14-16 in. of the roots—a tendency which was also very noticeable in all the barley series. The nitrate plants here did not show the crinoline effect which was so noticeable in the barley manured with nitrate, but the roots of the wheat receiving potash were very similar to the corresponding barley roots, for near the crown the roots of the potash plants were very woody and stood out exactly as described for barley, though to a less marked degree.

In the early stages of growth, seven weeks after sowing, there was a distinct tendency towards thickening at the ends of the long branched roots, and this tendency became more marked as growth proceeded. When the roots had attained a considerable length, it was found that they coiled up at the bottom of the pots, and the coiled up parts were always much thicker than the rest of the root, and also bore few or no laterals.

No 'white' roots appeared until seven weeks after sowing, when a few were found in the more heavily manured plants. Later, they were present in all the manurial series, but in no case did they retain for very long their unbranched character, the result being that long, branched, 'white' roots were found fairly frequently.

TABLE IV.

Wheat. (Sown February 21.)

Showing average number of 'white' roots at different dates.

Date of observation.	No. of weeks after sowing.	Unmanured.	NaNO ₃ .	Super-phosphate.	Super-phosphate + NaNO ₃ .	Super-phosphate + K ₂ SO ₄ .
April 13	7	1	2	2	1	3
April 27	9	2	2	5	5	6
May 11	11	3	4	7	8	8
June 1	14	9	7	13	12	8
June 15	16	18	16	14	18	16
July 13	20	2	3	2	0	2
August 24	26	0	0	0	0	0

The numbers of white roots reached their maximum about 16 weeks after sowing. The superphosphate plants were lowest with average number 14, while the unmanured and the superphosphate and nitrate plants were highest with 18. A certain number of white roots was found after this date, but when the plants were mature none was found in any of the series. Thus there is in wheat nothing to correspond to the sudden disappearance of white roots which occurs in barley about 11 weeks after sowing, for in wheat the decline in white root numbers coincides with the decrease in weight of the complete root system, whereas in barley the formation stops suddenly when the ratio between shoot and root growth begins to change.

Relative growth of Root and Shoot in Wheat. The general behaviour of the root and shoot of wheat at different periods of growth proved to be similar to that of barley, though the details varied with the different manurings (Table V).

For the first seven weeks growth was very slow and little difference in dry weight was observable either between roots and shoots or with different manures. During the next four weeks the amount of growth, as measured by dry weight, increased considerably, but the shoots did not seem to get away properly until after May 11, after 11 weeks' growth. The shoots increased steadily in weight till about a month before harvest, when a slackening of the rate of growth occurred except with superphosphate and nitrate applied separately. The roots, as in barley, reached their maximum long before harvest and then steadily lost weight in every case. The striking feature of the pot cultures of wheat was the close similarity in dry weight of the unmanured plants and those receiving nitrate only, this being obvious both with roots and shoots. Also, the shoots of all plants receiving superphosphate, whether with or without other manures, were almost identical in weight for 14 weeks, after which those receiving nitrate or potash in addition ran ahead of those with superphosphate alone. The maximum root growth was attained after about 16 weeks in all the manured plants, but the unmanured roots continued to increase in weight

TABLE V.
Dry Weights of Individual Wheat Plants.
Sown February 21, 1920.

Date.	Unmanured.			Sodium nitrate.			Superphosphate.			Superphosphate + Sodium nitrate.			Superphosphate + Potassium sulphate.		
	Shoot.	Root.	Total.	Shoot.	Root.	Total.	Shoot.	Root.	Total.	Shoot.	Root.	Total.	Shoot.	Root.	Total.
April 13	0.06	0.04	0.10	0.09	0.07	0.16	0.11	0.11	0.22	0.14	0.08	0.22	0.23	0.11	0.34
	0.08	0.07	0.15	0.10	0.06	0.16	0.24	0.12	0.36	0.23	0.10	0.33	0.19	0.11	0.30
April 27	0.12	0.13	0.25	0.25	0.22	0.47	0.58	0.58	1.16	0.52	0.34	0.86	0.50	0.35	0.85
	0.19	0.16	0.35	0.21	0.21	0.42	0.61	0.41	1.02	0.67	0.45	1.12	0.77	0.40	1.23
May 11	0.26	0.22	0.48	0.70	0.48	1.18	1.65	1.04	2.69	1.07	0.48	1.55	1.01	0.61	1.62
	0.33	0.31	0.64	0.45	0.39	0.84	1.55	1.80	3.35	1.64	1.32	2.96	1.76	0.98	2.74
June 1	3.01	1.14	4.15	3.12	1.21	4.33	7.97	4.25	12.22	6.89	2.15	9.04	9.70	4.31	14.01
	2.81	1.25	4.06	3.20	1.78	4.98	7.72	3.70	11.42	7.38	3.01	10.39	5.61	3.05	8.66
June 15	6.17	1.93	8.10	9.83	2.85	12.68	11.04	2.81	13.85	14.72	4.62	19.34	13.66	4.01	17.67
	8.12	3.05	11.17	9.16	2.50	11.65	11.24	5.33	16.57	13.07	3.10	16.17	10.18	4.93	21.11
July 13	14.52	3.39	17.91	14.85	2.61	17.46	13.46	2.91	16.37	19.33	2.25	21.58	20.48	3.04	23.52
	15.76	3.30	19.06	15.30	2.31	17.61	18.51	3.67	22.18	19.14	3.23	22.37	22.04	5.17	27.21
August 24	18.74	2.08	20.82	17.70	1.62	19.32	22.71	1.48	24.29	17.15	1.28	18.43	23.11	1.83	24.94
	14.30	1.08	15.38	23.54	1.62	25.16	21.18	2.87	24.05	20.66	1.06	21.72	—	—	—

for another month. Superphosphate applied alone tended to hasten the rise to a maximum, but in this case the fall did not begin to occur immediately. The roots and shoots of the nitrate plants closely followed

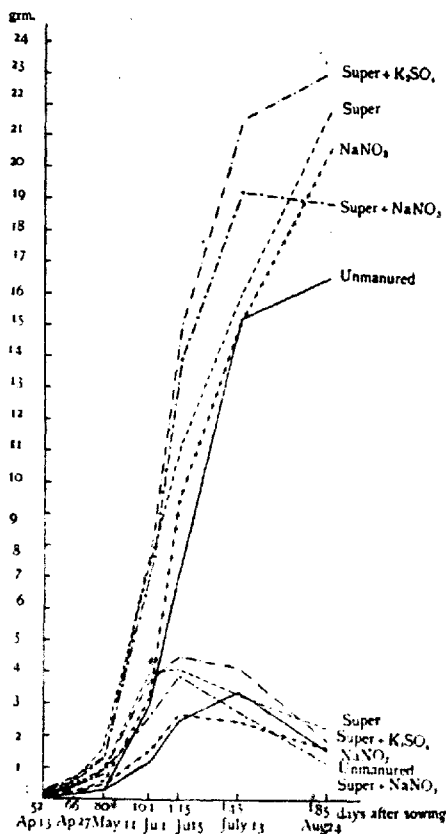


FIG. 3. *Wheat*, plants grown in pots. Dry weights of shoots, upper curves; dry weights of roots, lower curves. Mean of two plants in each case.)

those of the unmanured in weight throughout almost the whole period of growth, indicating that, under the experimental conditions at least, nitrate by itself exercised very little beneficial action on the growth of wheat. At harvest time the shoots of the nitrate plants were, however, heavier than the unmanured, probably because the effect of nitrate is to lengthen the period of growth, so that while the rate of growth began to slacken off in the unmanured plants about six weeks before harvest, in the nitrate plants growth continued steadily to the end.

In this case, again, maximum root development appeared to be attained almost simultaneously with the emergence of the ears from the sheaths, though with superphosphate this maximum was reached earlier, exactly as occurred with barley. With no manure, on the contrary, root growth continued after the ears had emerged, even though the latter event was somewhat later than in manured plants. As the two cereals followed one another so closely, it seems probable that the same explanation as to association of purely vegetable growth with increase of root weight will hold good in both instances.

In wheat the shoot/root ratio appeared to fall for about nine weeks, and though a slight rise took place in the next fortnight no rapid increase in

TABLE VI.

Shoot/Root Ratio. Wheat.

Date.	Unmanured.	Sodium nitrate.	Super-phosphate.	Super-phosphate + Sodium nitrate.	Super-phosphate + Potassium sulphate.
April 13	1.32	1.22	1.97	1.91	2.16
	1.18	1.51	2.04	2.23	1.72
April 27	0.90	1.16	1.51	1.56	1.42
	1.24	1.00	1.48	1.47	1.66
May 11	1.18	1.45	1.59	1.54	1.65
	1.05	1.16	1.92	1.25	1.80
June 1	2.64	2.57	1.88	3.21	2.25
	2.24	1.80	2.09	2.45	1.84
June 15	3.19	3.45	3.93	3.19	3.41
	2.66	3.69	2.11	4.22	3.28
July 13	4.28	5.70	4.63	8.60	6.75
	4.78	6.63	5.05	5.92	4.58
August 24	9.01	10.95	14.34	13.43	12.66
	13.24	14.56	7.37	19.54	—

the ratio occurred till after this time. The effect of the superphosphate in increasing the relative proportion of shoot to root was evident at a very early date, all the ratios for the first 11 weeks being above those for unmanured and nitrate plants, in which it was not much above unity. As time went on the effect of the nitrate, especially when combined with superphosphate, became more marked, showing that the shoot increased relatively more rapidly than the root. The unmanured and superphosphate plants, however, showed almost identical shoot/root ratios after the earlier period was past, indicating that the phosphate gave equal encouragement to both organs when the initial stages of growth were over (Fig. 4 and Table VI). The change in the curve of the shoot/root ratio was less sharply marked in wheat than in barley, and took place considerably earlier. This may be accounted for by the fact that wheat is typically autumn sown, is used to coping with unfavourable weather conditions, and can start into active growth with comparatively low temperature. The change in the shoot/root ratio of wheat began as soon as the mean maximum temperature

reached 55° F. Barley, on the other hand, is typically spring sown and needs a higher temperature before the onset of active growth can occur, a mean maximum of over 60° F. being needed in this case.

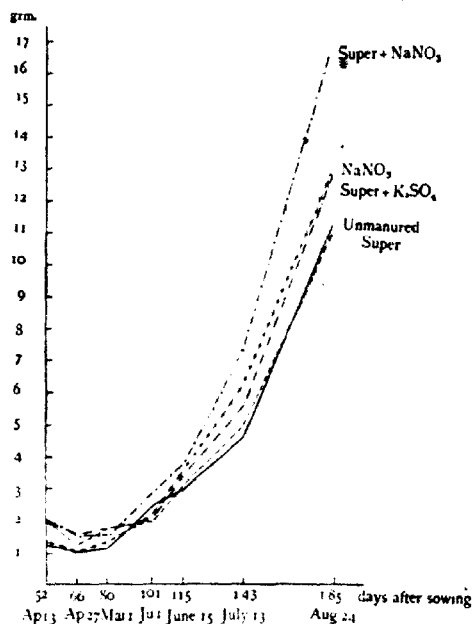


FIG. 4. *Wheat*, shoot root ratio.

Field Trials.

Wheat. Growth of Roots in the Field.

Owing to pressure of laboratory work it was not possible to wash out the wheat roots from Rothamsted soil. Ten Eyck,¹ however, found that the main roots grew almost vertically downwards, giving off numerous branches which made a fine network in the upper part of the soil, while the deeper roots also gave off many branches. The upper roots curved outwards before striking down into the soil, acting somewhat as brace roots, a condition of things that recalls the stiffening of the barley roots near the crown when potash manures were used.

DISCUSSION.

At the outset of the experiment it was anticipated that some information might be gained as to the effect of various manures on the depth of rooting of wheat and barley. Shepperd² found in his field experiments that the

¹ Ten Eyck : loc. cit.

² Shepperd : loc. cit.

*system of rooting in wheat is vertical and that wheat roots may normally run down to a depth of 4 ft., whereas barley and oats tend to show a comparatively light and shallow growth. This is common experience, but the factors which cause the difference need examination.

In the pot cultures both wheat and barley behaved in the same way with regard to the depth of penetration. The first-formed roots, branching freely, soon reached the bottom of the pots, and, continuing their growth with much less branching, coiled round the bottom and gradually formed an interlaced mat of roots in the larger plants. The pots were 14 in. deep, and for about that distance strong concentration of laterals occurred, no difference being observable between the two species. The consistent results obtained showed that the limiting factor to strong lateral production in this case was the depth of the pots; also the total length of the roots when uncoiled was much the same in both plants. It must be borne in mind that in these pot-culture experiments the soil was carefully sifted and shaken into position, but not rammed. Watering was carefully and regularly done, so that water-logging did not occur, and thus the plants were growing in a friable, well-aerated, moist soil, and did not suffer either from drought or excess of moisture. Under these favourable conditions heavy root growth was made, and barley proved able to develop as deep and strong a root system as wheat within the limits imposed by the pot. In both cases the unmanured plants had weaker roots than the manured, except that sodium nitrate failed to improve the root growth of wheat.

The field experiments, with barley, revealed quite a different state of affairs. The Rothamsted soil is heavy, and on the plots examined the tilth was poor, so that below the first inch or two the soil became very consolidated and about five inches down passed into clayey undisturbed subsoil. Under these conditions little tillering of the barley shoots occurred, and the roots when washed out were very thin and poverty stricken. The number of roots springing from each seed was small, they were thin and but slightly branched, and did not penetrate very far into the soil in a vertical direction. Instead they tended to go off at an angle, to run more or less horizontally with a downward inclination, and to take advantage of any easier passage, such as that offered by a piece of straw in the soil, a worm track, or the cavity struck out by the underground part of a strong growing weed such as *Convolvulus arvensis* or *Cirsium arvense*. The whole root system when washed out was very small and offered no comparison with that of the pot plants. The results were the same for unmanured and superphosphate plots at the time the barley was ripe and from similarly manured plots on which the barley was still green. Practical experience suggests that the roots would have been somewhat larger at an earlier stage, but the shoots showed conclusively how poor the root must have been even at its best.

It might be objected that when the roots were washed out *in situ*

many were broken off and overlooked, but that this was not the case was proved by the other experiment, previously described, in which blocks of soil containing the whole root system were removed and soaked and washed out under conditions allowing of the recovery of all broken pieces. Even under these circumstances the longest roots were only 9 in. long, and the results compared closely with those obtained in the field.

The results of these experiments may be considered from two aspects:

1. Influence of environmental conditions, other than manuring, upon root growth.

2. Influence of different types of manuring on root growth.

1. *Influence of environmental conditions, other than manuring, upon root growth.* The general type of the root system seems to be a more or less constant character of the species concerned, but wide variations within the type may occur through the influence of soil factors.

The environmental conditions presented by the field and pot cultures were so radically different that it is not surprising to find the root systems of both wheat and barley developed differently in each case. The lack of penetration of the barley roots into the hard field subsoil is a well-known phenomenon observable in many plants. Markle¹ indicates that with desert plants a layer of soil that is difficult of penetration may cause much distortion of roots entering it. Also, roots will often turn abruptly from a layer of clay and follow a thin layer of sand or fine gravel containing much less water but more easily penetrable. This was paralleled by the behaviour of barley roots in utilizing worm-tracks or other passages when opportunity offered.

Shepperd found with maize that the roots penetrated less deeply when a layer of shale was present under the surface soil, rendering the lower layers dry and uninviting to plant roots.

Associated with the question of penetrability is that of the aeration of the soil and its effect on root growth. In experiments in India Mr. and Mrs. Howard² found that for adequate development the roots must have abundant 'room' in the soil for rapid growth and the space must be adequately ventilated. The rooting habit was greatly influenced by the soil conditions. Linseed, when grown on alluvium, was shallow rooted, but on deeper soils penetrated much farther. This last effect was attributed to the cracking of the upper soil, whereby the subsoil was more efficiently aerated and deeper rooting rendered possible. Similar results were obtained by Cannon,³ who states that deep-rooted plants are less dependent on good

¹ Markle, M. S. (1917): Root Systems of Certain Desert Plants. *Bot. Gaz.*, lxiv, pp. 177-205.

² Howard, A. and G. L. C. (1917): The Economic Significance of the Root Development of Agricultural Crops. *Agr. Jour., India, Special Indian Sci. Congress Number*, pp. 17-28.

³ Cannon, W. A. (1915): On the Relation of Root Growth and Development to the Temperature and Aeration of the Soil. *Amer. Journ. Bot.*, ii, No. 5, pp. 211-24.

aeration than shallow-rooted. The latter tend to branch most freely near the surface of the ground, where aeration is good and soil temperature is favourable, the deeper rooted plants being less sharply limited in growth by the soil conditions. These conclusions were fully borne out by the Rothamsted results. Barley, typically shallow-rooted, responded at once to the favourable conditions of penetrability and aeration under pot-culture conditions, and developed a root system which corresponded closely with that of the typically deep-rooting wheat. In the field, on the contrary, where the soil was badly aerated and difficult to penetrate, the barley roots were short and very poorly developed, whereas those of the wheat were apparently able to strike more deeply and develop more strongly.

Water and the available food-supply have considerable influence on development. Von Seelhorst working with rye,¹ wheat, barley, &c., found that when liberally fertilized the plants have a larger root system, the roots descending deeper into the soil and thus being able to withstand drought better.

Cannon² states that soil temperature is another vital factor in determining the type of root growth, as roots which occupy different soil horizons in the same habitat are exposed to widely different temperatures at all seasons. In working with desert plants he found that the growth-rate of roots varies with temperature, that of deep-rooted plants being more rapid at all temperatures than that of shallow-rooted. No temperature readings were taken during the Rothamsted experiments, but it may safely be assumed that as the pots were relatively small and exposed on all sides to the air, the temperature of the soil at the bottom of a pot approximated more closely to that at the top than the temperature 14 in. deep in the soil to that of the surface soil. This being the case the temperature conditions were more favourable to the growth of roots in the pots than in the field, and this, added to the other favouring conditions of aeration and penetrability, probably helped the shallow-rooting barley to strike deeply and develop a strongly branched root system.

Modestov³ carried out experiments on the root development of races of the same species, and found that different races of wheat and oats grown under similar conditions showed essential differences in the length and weight of their roots. In oats the length of the roots was in inverse proportion to the time of ripening, deeper rooting resulting in later ripening. The idea suggests itself that possibly this may hold good outside the limits of a single species, as shallow-rooted crops like barley

¹ Seelhorst, C. von (1902): Beobachtungen über die Zahl und den Tiefgang der Wurzeln verschiedener Pflanzen bei verschiedener Düngung des Bodens. Jour. Landw., 1, No. 1, pp. 91-104.

² Cannon, W. A. (1915): loc. cit.

³ Modestov, A. P.: Les racines des plantes herbacées. Kornevaia sistema Traviants'kh. Kastenii., Moscow, I. N. Kushnerov, 1915, No. 1, pp. 223. See Exp. Stat. Rec., vol. xxxvi, 1917, p. 223.

tend to have a shorter growing season than deep-rooted crops like wheat.

2. *Influence of different types of manuring on root growth.* The effect of manuring on root growth in pot culture has been considered in the earlier part of the paper, but may be summarized briefly here.

In *barley* the use of artificial fertilizers, whether containing K, N, or P, induce a great increase in root development, this being most marked in the presence of sodium nitrate, either alone or in conjunction with superphosphate. With *wheat*, on the contrary, nitrogen manuring failed to bring about any increase, while the improvement with superphosphate and superphosphate + potash was far less evident than in *barley*.

In *barley*, again, the number of 'white roots' was distinctly increased by the use of superphosphate, but with *wheat* the ultimate number of these roots was much the same whatever the system of manuring. It is not possible to say definitely whether these results would hold good under normal conditions of cultivation, as the soil conditions presented in pot cultures are so peculiarly favourable that it would be unfair to draw from them any conclusions with regard to similar experiments in the field.

Apart from the question of depth of rooting the formation and function of the 'white roots' offer a promising field for inquiry. A comparison of the anatomical structure of the ordinary and white roots is being made by one of us, and discussion of the subject will therefore be postponed till a later paper, when more information will be available.

Studies in the Physiology of Parasitism. VII. Infection of *Berberis vulgaris* by Sporidia of *Puccinia graminis*.

BY

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With nineteen Figures in the Text.

A GOOD deal of attention has of late been paid to the mode of infection of host plants by certain fungi. Blackman and Welsford (1) showed that in the case of *Botrytis cinerea* the entry of the parasite was not brought about by the dissolution of the cuticle of the host, as previous workers had supposed, but was due to the mechanical pressure exerted by the germ tube. Dey (2), working with *Colletotrichum lindemuthianum*, obtained similar results. The previous work bearing on the subject of cuticular penetration is referred to and summarized in these two papers and need not be referred to here.

The present investigation was carried out to determine whether in such a widely different form as *Puccinia graminis*, Pers. entry into the host was brought about in the same way.

The mode of infection of the wheat plant by uredospores of *P. graminis* through the stomata has already been carefully worked out (3). This work followed on a similar investigation by Marshall Ward dealing with the related form *P. dispersa*, Erikss. (4). An investigation of the entry into the host of the aecidiospores and uredospores of a number of other rusts was carried out by Miss Gibson (5). Little, however, appears to have been done in connexion with the details of sporidial infection in any rust fungus. Eriksson (6) refers to the mode of entry of the sporidia of *P. Malvacearum* into the hollyhock in the following terms: "Wahrscheinlich infolge einer auflösenden Einwirkung des Körpers selbst auf die Epidermiswand bildet sich an dem Kontaktpunktchen ein sehr feines, kaum sichtbares Loch, durch das sich der Inhalt des Körpers hineinergiesst" (p. 75).

METHODS.

In April 1920 rusted wheat stubble was collected from a clover field at Milton in Pembrokeshire. It comprised sheath and stem, very badly rusted with teleutosori of *P. graminis*, of the crop that had been harvested in 1919. During the winter it had of course been fully exposed to the weather. The teleutospores proved viable. This was expected, for sprouting barberry hedges in the vicinity were at the time showing the aecidial stage of the rust. A portion of the straw was kept at ordinary laboratory temperature and a portion in cold storage at a temperature a little above freezing-point. The former gave excellent germinations up to June, and then, as the spores germinated less abundantly, the material that had been kept cool was used. Up to the end of July the germination of these teleutospores was still very good and the sporidia remained capable of infecting the barberry.

To obtain sporidia for study, small pieces of the washed straw containing teleutosori were placed in hanging 'drops' of water on cover-slips with the teleutosori facing downwards. These were supported on rings over sterile slides on which were placed drops of sterile tap-water, of very dilute turnip-juice, or of barberry extract. The latter was prepared by boiling 50 gm. of barberry shoots in 1,000 c.c. of water for half an hour, filtering, and sterilizing. These slides were kept in a moist chamber at laboratory temperature. It was found that better results were obtained by using complete sori in this way than by using scrapings of the sori, giving separated teleutospores. The sporidia, formed in the moist atmosphere of the hanging drop (7), fell on to the liquid on the slide and germinated readily. These cultures were contaminated with bacteria and other organisms which spoiled the cultures after about three days. Numerous attempts to grow the sporidia on agar media failed for this reason.

For demonstrating the presence of a mucilaginous sheath, dilute aqueous gentian violet gave better results than Indian ink or collargol. This investment was best seen in the germinations in dilute turnip-juice. Germinating sporidia were also fixed and stained with picro-nigrosin, or fixed in Flemming's fluid and stained in iron-alum-haematoxylin and the triple combination, in order to observe the nuclear details.

For infection work, plants of *Berberis vulgaris* in pots were obtained and placed in a greenhouse. They were pruned back heavily and the young developing shoots were used for infections. Cut shoots placed in water in sterile glass jars loosely plugged with cotton-wool were tried, but abandoned in favour of shoots still attached to the plant. The shoots were well watered with a fine spray, and then small pieces of the rusted straw, which had been thoroughly washed and then soaked for half an hour in sterile water were, placed on the upper surface of the young leaves.

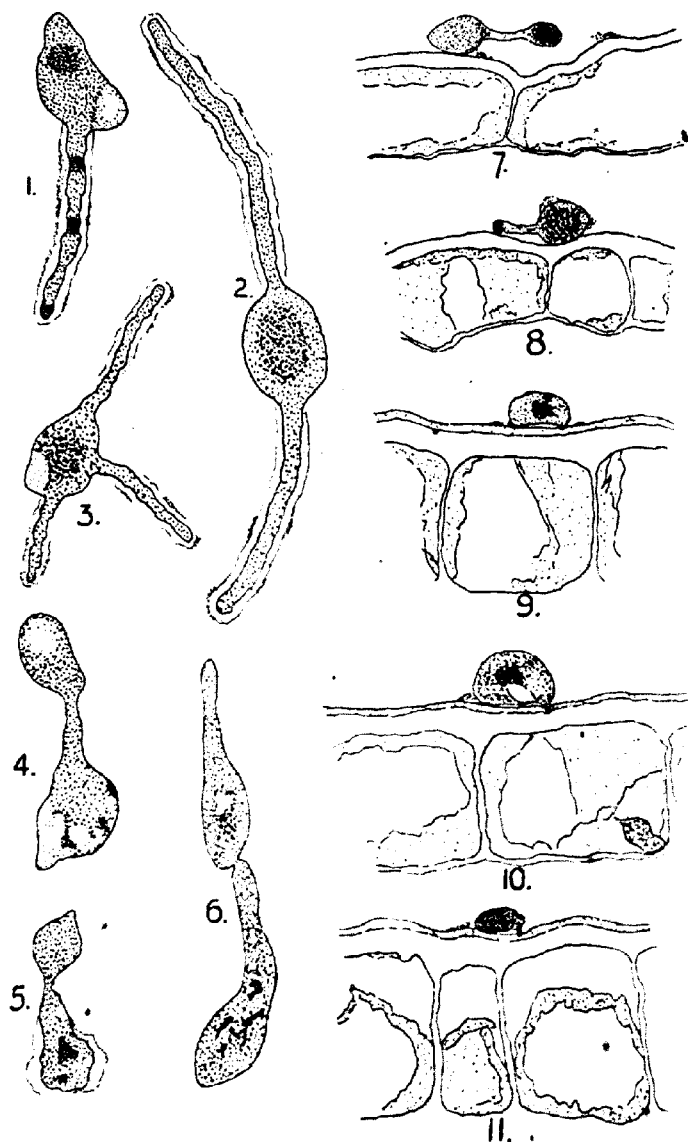
The infected shoots were then enclosed in glass or mica cylinders plugged with cotton-wool. Better germinations and more abundant infections were obtained in this way than by sowing teleutospores on the leaves, although infections were obtained by the latter means. Attempts to get infection of mature leaves invariably failed.

Small pieces of the infected leaves were fixed at intervals in acetic alcohol (absolute alcohol three parts, glacial acetic acid one part), and in Flemming's strong solution diluted with an equal volume of water. Both gave good results. The material was embedded in the usual way and was cut into sections $4\ \mu$ thick. In the clearing process, satisfactory results were obtained from the cedar-wood oil method, in which the dehydrated material was placed in absolute alcohol floating on cedar-wood oil, whence it gradually passed into the latter liquid under the action of gravity.

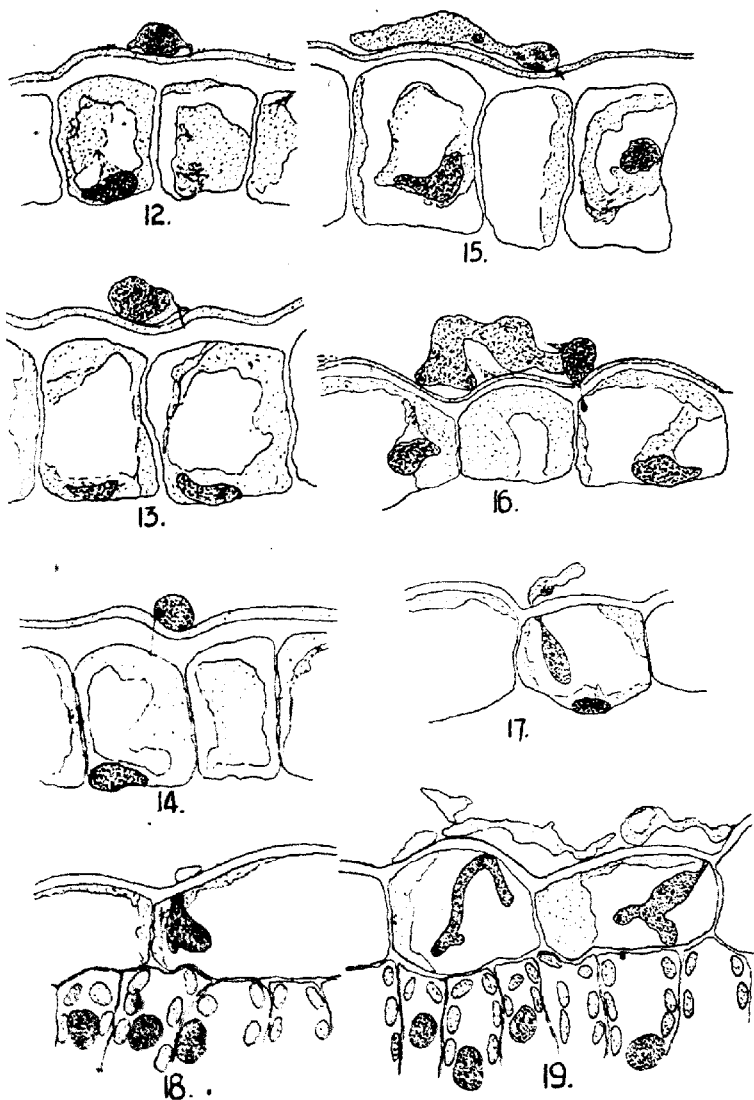
For demonstrating the early stages of infection the best details were given by iron-alum-haematoxylin followed by Sudan III. Such sections mounted in glycerin jelly showed the cuticle very sharply stained. Scharlach red and Congo red in place of the Sudan III gave almost as good results. Sections stained with Congo red alone and mounted in euparal also showed up well. For the latter stages of infection, the safranin-gentian violet-orange G combination gave the best results.

OBSERVATIONS.

Germinations on the slide. Germination of the teleutospores was rapid. Promycelia were to be seen after 5 to 6 hours and sporidia were obtained on the slide after 12 to 15 hours. These at once germinated by pushing out a germ tube (Fig. 1); sometimes two (Fig. 2) or even three (Fig. 3) such tubes were produced. It was early found that the germinating sporidia adhered readily to the slide and could be stained, washed, &c., without loss. The adhesion is due to the fact that the germ tubes possess a mucilaginous sheath similar to that of *Botrytis cinerea*. This stains faintly with dilute aqueous gentian violet. At the edges of the sheath, at a distance from the main wall of the germ tube, aggregations of particles further emphasize the presence of the sheath (Figs. 1, 2, and 3). The germ tube may continue to elongate, and becomes septate; in one case it attained a length of over 1 mm. Frequently, after elongating slightly, the germ tube swells into a vesicle (Figs. 4 and 5). In some cases the vesicle was large and in contact with the glass slide and was therefore of the nature of an appressorium; around such an appressorium a mucilaginous investment may or may not be observed (Fig. 5). From the vesicle may arise a second germ tube (Fig. 6). It was generally found that the production of long germ tubes was most frequent when the sporidia were in water. In the nutrient media the production of vesicles was more common, and in the barberry extract these vesicles were often very irregular in shape.



FIGS. 1-11.



FIGS. 12-19.

When the germinated sporidia are fixed and stained the remnants of the mucilaginous covering appear as irregular threads and granules (Figs. 7, 11, 16). Frequently masses of sporidia adhering together were observed, both in the germinations on the slide and on the barberry leaf. It was not possible to trace any transition from a narrow well-defined wall to a wide indefinite gelatinous investment as described for *Botrytis cinerea* (1, Fig. 3).

Penetration of the host. Sporidia were observed to have penetrated into the epidermal cells of the barberry leaf in 20 hours. In fixed material, sporidia were seen closely adherent to the leaf surface 17 hours after the teleutosori had been placed on the leaf. No mucilaginous sheath was demonstrated in the case of ungerminated sporidia, but it is possible that a certain amount of gelatinization of the spore wall may take place, anchoring the sporidium to the cuticle. Irregular threads and granules are commonly seen in the immediate neighbourhood of the sporidia and may represent the remains of this mucilage.

On the leaf, germination may result in the production of a definite germ tube. This may develop quickly into a vesicle (Fig. 7), or may grow on for some distance before it reaches the cuticle (Fig. 8) and becomes closely adpressed to the surface. In other cases the germ tube is seen to ramify and grow over the surface for a considerable distance without forming an appressorium.

Instead of producing a definite germ tube, it frequently happens that from the end of the sporidium a short beak-like infection hypha is put out (Fig. 9) which presses closely on the cuticle and may produce a slight indentation in it (Fig. 10). In some cases the growth of the beak-like infection hypha forces the end of the sporidium away from the leaf surface (Fig. 11); the tip of this hypha is densely protoplasmic. The hypha then produces an extremely fine style-like structure which penetrates the wall of the epidermal cell (Figs. 13 and 14). In appearance and in its size relative to the body of the sporidium, it reminds one of the proboscis of an insect. This style is pushed through the cuticle and underlying cellulose layers of the epidermal wall (Figs. 13, 14, and 15). A very careful study of the cuticle at the point of entry was made, but no swelling or any sign of alteration in its staining properties could be detected. This penetration of the wall by the stylar outgrowth is rendered possible by the firm adhesion to the cuticular surface which results from the mucilaginous investment of the germ tube. It therefore seems clear that the penetration of the cuticle is brought about mechanically by the pressure exerted by the style-like apex of the infection hypha. No alteration in the nuclei, when these were present in the epidermal cells, or in the cellulose layers underlying the cuticle, could in any case be detected at this stage.

In the barberry leaf, no stomata are present in the upper surface of the leaf, so that entrance into the host always takes place by puncture of the

cuticle. A parallel series of infections with *Puccinia Malvacearum*, Mont., was made on leaves of hollyhock, in which stomata are present on the upper surface of the leaf. Eriksson (6, p. 75) states that in no case was he able to observe the entry of a germ tube through a stoma. In one case the beak-like projection of a germinating sporidium was found inserted into a stomatal pore, although, in agreement with Eriksson, the mode of entry was by a penetration of the cuticle in all other cases.

After penetrating the epidermal wall, the tip of the infection hypha at once begins to swell (Fig. 16). The swelling enlarges and becomes an elongated vesicle (Fig. 17), which branches (Fig. 19) and gives rise to the uninucleate mycelium which invades the host tissue.

In conclusion, the author wishes to express to Professor V. H. Blackman his gratitude for helpful advice and criticism; also to Dr. S. G. Paine, and Mr. J. F. Dastur, of India, for help in connexion with the work.

SUMMARY.

The infection of *Berberis vulgaris* by the sporidia of *Puccinia graminis* has been studied.

In the germinating sporidia, a mucilaginous investment of the germ tube can be shown to be present.

- Penetration of the cuticle is brought about by means of a very fine style-like infection hypha, which may be put out either from the end of a definite germ tube or from a short beak-like outgrowth of the sporidium.

There is no evidence of any chemical action upon the cuticle. The puncture of the cuticle appears to be brought about solely by the mechanical pressure exerted by the infection hypha as it develops from the germinating sporidium or germ tube. The sporidial beak or germ tube from which the infecting style grows is firmly fixed to the leaf surface by means of a mucilaginous investment.

As soon as the infection style has penetrated through the epidermal wall it swells into a vesicle from which the mycelium arises.

The entry of the parasite causes at first no visible alteration of the cell contents of the host plant.

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5. GIBSON, C. M. (1904): Notes on Infection Experiments with Various Uredineae. New Phytol., iii, 184.
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EXPLANATION OF FIGURES.

The host tissue in every case is that of the leaf of *Berberis vulgaris*.

All figures were drawn with the camera lucida under a Koristka $\frac{1}{2}$ -inch semi-apochromatic oil-immersion objective and No. 8 eyepiece, except Figs. 1-3, which were drawn under a Leitz $\frac{1}{2}$ -inch oil-immersion objective and No. 12 eyepiece.

Fig. 1. Sporidium germinating on slide and producing a single germ tube with a mucilaginous sheath. Drawn from living material stained with weak aqueous gentian violet. $\times 2,000$.

Fig. 2. Germinating sporidium producing two germ tubes with mucilaginous sheaths; material as in Fig. 1. $\times 2,000$.

Fig. 3. Germinating sporidium producing three germ tubes; material as in Fig. 1. $\times 2,000$.

Fig. 4. Germinating sporidium producing a vesicle; material as in Fig. 1. $\times 1,560$.

Fig. 5. Germinating sporidium producing vesicle with a mucilaginous investment; material as in Fig. 1. $\times 1,560$.

Fig. 6. Sporidium germinating and producing a vesicle which is developing a germ tube above; material as in Fig. 1. $\times 1,560$.

Fig. 7. Sporidium germinating on barberry leaf and producing a vesicle. $\times 1,560$.

Fig. 8. Sporidium on barberry leaf, germinating with production of a simple germ tube, the tip of which is adpressed to the surface of the leaf. $\times 1,560$.

Fig. 9. Sporidium attached to leaf and producing a beak-like protuberance at one end. $\times 1,560$.

Fig. 10. Beak-like projection of sporidium producing an indentation of the cuticle. $\times 1,560$.

Fig. 11. Outgrowth from sporidium pressing upon the cuticle and forcing up the end of the sporidium. $\times 1,560$.

Fig. 12. Sporidium producing a longer beak-like process with its densely protoplasmic tip closely pressed against the cuticle. $\times 1,560$.

Fig. 13. Sporidium producing a style-like infection hypha which has pierced the cuticle. The section is cut in such a way that the actual connexion between the style and the sporidium is not shown. $\times 1,560$.

Fig. 14. Sporidium showing the infection hypha which has penetrated the whole thickness of the epidermal wall. $\times 1,560$.

Fig. 15. Infection hypha, developed from a germ tube, which has pierced the cuticle. $\times 1,560$.

Fig. 16. Germination of sporidium has led to the formation of irregular vesicles. An infection style has penetrated the epidermal cell and is beginning to swell at the tip. $\times 1,560$.

Fig. 17. A later stage of penetration. The vesicle in the epidermal cell has increased in size. $\times 1,560$.

Fig. 18. Larger vesicle showing three nuclei. $\times 1,560$.

Fig. 19. Two epidermal cells which have been invaded, showing the branching of the mycelium arising from the infection vesicle. The shrivelled remains of the germinated sporidia are to be seen on the leaf surface. $\times 1,560$.

Anatomy of the Seedling and Young Plant of *Macrozamia Fraseri*.

BY

E. J. HATFIELD.

With Plate XXII and eight Figures in the Text.

THE anatomy of the genus *Macrozamia* has received attention at the hands of several botanists. In 1902 Miss Robertson¹ gave a summary of the chief results derived from studies of the genus up to that date.

The anatomy of the species *M. Fraseri* is of peculiar interest, for it was the discovery by Worsdell² of secondary and of tertiary 'inverted' anomalous rings of xylem in the mature stem which led to the formulation of the Medullosean theory of the origin of the Cycads. In view of the importance that has been attached to the adult anatomy, Dr. Thomas suggested that an investigation of young plants recently obtained by her in Australia seemed desirable.

The examination of germinating seeds revealed a structure quite comparable in its main features with that recorded for other seedlings of the genus^{3, 4, 5, 6} and upon which the generalized account of Cycadean seedling anatomy given by Coulter and Chamberlain is founded; but, if transition phenomena be figured in the diagrammatic form given by them, the transition in *Macrozamia Fraseri* corresponds rather to that given for *Ginkgo Gnetum*, and *Araucaria* than to those representing the Cycadean genera. In the young plants, while some of the structures found in the adult are absent—e.g. pith bundles, 'inverted' anomalous rings, &c.—a hitherto undescribed anomaly is revealed in the epicotyledonary region. This is a gradual tangential extension of the cambium, and of the elements to which it gives rise, until a vascular cylinder is developed, which resembles in this feature the structure found by Dr. Stopes⁷ in the

¹ Robertson: Proceedings of the Cambridge Philosophical Society, vol. xii, Part I.

² Worsdell: Ann. Bot., vol. x, 1896.

³ Dorey: Vascular Anatomy of the Seedling of *Mitrasac calycina*. Bot. Gazette, vol. xlvii, 1909.

⁴ Dorey: The Seedling of *Crataegus*. Bot. Gazette, vol. xvi, 1908.

⁵ Thiessen: Anatomy of the Seedling of *Pison altic*. Ibid., 1908.

⁶ Hill and de Friaide: Seedling Structure of Gymnosperms. III. Ann. Bot., vol. xxiii, 1909.

⁷ Stopes: Catalogue of the Cretaceous Flora, Part II, p. 314 et seq.

[Annals of Botany, Vol. XXXV, No. CXL, October, 1921.]

Cretaceous fossil *Colymbites Edwardsii*. Further, an examination of the stem apex reveals a very definite and extensive increase of parenchymatous tissue, as the result of the activity of a meristematic 'growth zone' which cuts off pith and cortical tissues by cell-division in the tangential plane only. It seems likely that this method of growth has some bearing on the phenomenon of tangential extension found in older portions of the young stem.

Finally, the structures observed strongly indicate that the phenomenon of 'girdling' is not a new feature peculiar to the Cycadales, but is rather the modification of a normal leaf-supply due to two factors, (1) the telescoping of the axis, and (2) the activity of the growth zone referred to above.

Material. This was found in a sandy Cycad thicket near Fremantle, in Western Australia, and collected by Dr. E. N. Thomas during the visit of the British Association to Australia in 1914. It consisted of seeds just beginning germination; also of young plants of tubby habit, which had borne, on the average, some twenty leaves (Plate XXII, Fig. I). These plants were characterized by a very swollen hypocotyl and an extremely long tap-root (at least 60 cm. in length). There is no evidence of any decay of the tap-root, and it would seem unlikely that so strong and well established an organ should be discarded later by the plant.¹ It is possible that changes in the habit of these plants, induced by conditions of culture in pots, have led to the idea expressed by English writers that the roots of mature Cycad plants are wholly adventitious. This view finds support in the fact that a three-year-old seedling of *M. Fraseri* grown in England had a short and branching tap-root, a condition not paralleled in any specimen collected in the field.

Plants grown from seed in England produced some ten leaves in three years; the 'tubby' plants (Plate XXII, Fig. I) collected in Australia in 1914 had borne about twenty leaves; it would therefore seem that they were several years old. But no growth stages between these and the germinating seeds were found. The reason for this, in the absence of continuous observations in the field, could not be discovered. Work of Professor Pearson,² which shows that the seedlings of *Welwitschia mirabilis* are probably only able to establish themselves in the wet seasons which alternate with several practically rainless years, is suggestive. Further stages in the development of the seedling were provided by plants grown at Bedford College from seeds brought home in 1914.

Methods. The germinating embryos were embedded in paraffin and examined in the usual way. For the young plants, which were large and complicated in structure, hand-sections were at first resorted to, in combination with series of thick slices, which were cleared with eau de Javelle and stained with acid fuchsin in the manner advocated by Matte³ and used later

¹ Worsdell: Structure and Origin of the Cycadaceae. Ann. Bot., vol. xx, No. lxxviii, 1906.

² Pearson: Proceedings of the Royal Society.

³ Matte: Recherches sur l'appareil libéro-ligneux des Cycadees.

by Marsh.¹ These being found insufficient to elucidate the finer details of structure, serial sections were then prepared on the Jung microtome, sometimes with the aid of a freezing apparatus. It was, however, still found impossible to elucidate some points in the anatomy, owing to the exceeding complication of the structure, and the absence, which these methods entail, of any good system of orientating the series. Finally, the plants were divided up, and their successive portions embedded in paraffin. This method proved very successful if sufficient time—about a month—were allowed for penetration. By the use of the Minot microtome longitudinal and transverse serial sections of practically every portion of the plant were thus obtained. Staining was generally done upon the slide, and gentian violet and Bismarck brown was the most usual combination used. In some cases, for coarse details of structure, the staining was done under wax with eosin.

I. SEED AND SEEDLING.

The seeds—3.5 cm. long and 2.5 cm. broad—contain embryos with closely adpressed, partially fused, hypogeal cotyledons of unequal length, the petioles forming below a cotyledonary tube.

On germination, disorganized coleorhiza, root, and lastly plumule emerge in the sequence given, and in the manner described for other Cycadean seedlings.² As the plumular axis grows, and the base of the first plumular leaf rapidly expands, the size of the opening in the cotyledonary tube by which the plumule is emerging is actively increased, on the side adjacent to the leaf midrib, by the action of a phellogen; this cuts off from the side of each cotyledonary petiole successive layers of cells, which become disorganized, and so give the plumular axis room to emerge and expand.

Anatomy: (a) Mucilage canals. Each cotyledon is traversed by two roughly parallel rows of mucilage canals—the one row within, and the other outside, the row of vascular bundles. These are reduced in the petiole, by anastomoses firstly between the canals of a row, and next between those of outer and inner rows, to a series of about five ducts, which alternate with the leaf-traces. New canals may arise lysigenously at any point in the cotyledon. Tannin-containing cells are numerous in the lower portion of the cotyledon, though absent from its apex. These occur in or immediately beneath the epidermal layer.

(b) Vascular system. The cotyledons show that parallel dichotomizing venation characteristic of Cycadean seedlings. In this species six mesarch traces occur, as a rule, in the middle region of the cotyledon—reducing

¹ Marsh: Notes on the Anatomy of *Stangeria paradoxa*. New Phyt., vol. xiii, Nos. 1 and 2, 1914.

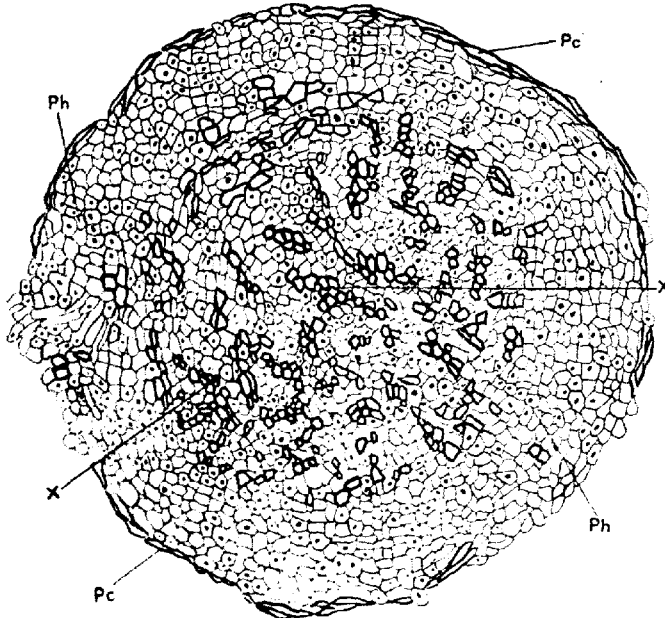
² Dorey: The Seedling of *Ceratophyllum*. Bot. Gazette, vol. xlvi, 1908, p. 46.

to four by fusion above the cotyledonary node. The strands are prevailingly mesarch throughout the greater portion of their length, becoming endarch or nearly so at the node. The protoxylem is in seriation with the centripetal metaxylem, and usually there is some parenchyma separating centripetal and centrifugal woods. There seems to be a tendency for the first elements of the centripetal xylem to develop on the flanks—i.e. right and left—of the protoxylem, and then, later, behind it. In the younger seedlings, where germination has only just started, no cambium is visible in the cotyledonary traces, and the structure is that described above. In older plants there is an obvious cambium, which is active, adding secondary xylem in the usual way. It would thus seem certain that a few elements of the centrifugal xylem of the cotyledons are primary in origin—a conclusion which agrees with that arrived at by Marsh¹ from an examination of the foliar traces of *Stangeria paradoxa*: 'At the base we have a centrifugal xylem not arranged in rows; . . . this portion is probably primary, and thus connects up the Cycadean foliar trace with the truly mesarch bundles of the Cycadofilicales.'

(c) *Plumule*. The plumule is enclosed in the cotyledonary tube during the earlier stages of its development. The axis is extremely short, and bears its earliest leaves almost, but not quite, opposite each other (Text-fig. 3, A). Each successive leaf, as it arises, misses by a very little being exactly opposite the previously formed one, and so, gradually, the typical spiral leaf-arrangement which characterizes the older plant is evolved. When very young, the leaves are hooded, fleshy structures, consisting of parenchymatous cells; as they develop, the lower portion becomes hollow, enclosing the next formed leaf by its two flanks. Apart from the leaf-traces, there is no development of xylem in the epicotyl of the seedling; it consists solely of parenchymatous tissue, which, shortly below the growing-point, is separated into pith and cortex by a dome-shaped mass of extremely meristematic tissue—the procambium.

(d) *Hypocotyl*. In the hypocotyl, a very short distance below the cotyledonary node, the vascular cylinder is a protostele (Text-fig. 1). This is circular in transverse section in the younger seedlings, and consists of a central portion made up of primary xylem elements and parenchymatous tissue surrounded by protophloem, the whole enclosed by a pericycle of fibrous elements with lignified walls (Text-fig. 1, *Pr.*). This vascular cylinder is exceedingly short. In the direction of the root the xylem elements of the central portion of the stele are seen to separate and group themselves round two centres, at opposite ends of a diagonal, to form a diarch root with its two poles situated in the cotyledonary plane; at the same time the phloem becomes concentrated into two masses in the intercotyledonary plane.

¹ Marsh, loc. cit.



TEXT-FIG. 1. Transverse section of protosteel in hypocotyl of young seedling. $\times 120$.
X. = xylem; Ph. = phloem; Pc. = pericyclic fibres.

(c) *Transition.* The course of the leaf-traces and the transition phenomena agree very closely in all the seedlings studied, and are perhaps best explained by reference to one particular case.

In a seedling in which germination has reached the stage shown in Text-fig. 2, three leaf primordia are to be distinguished. The first plumular leaf contains, at its junction with the stem, six mesarch bundles, arranged in an open arc (Text-fig. 3, A. L_1^1 L_1^6). These soon reduce, by the fusion of two, to five, and this number continues throughout its petiole, the traces becoming exarch towards its distal end. Just below the insertion of the first pinna, these five entirely exarch traces are reduced to four by the fusion of the second and fourth anteriorly to the third and central trace.

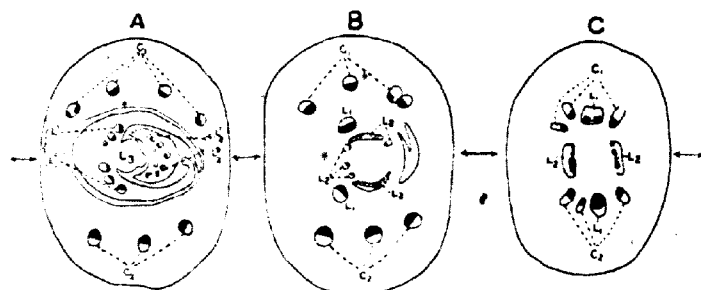
In the second leaf of the plumular bud nine



TEXT-FIG. 2. Seedling of *Macrozamia Fraseri*. Somewhat reduced size. Ap. = Apogeous root.

traces are eventually differentiated in the basal region (Text-fig. 3, $L_2^1-L_2^3$). These are represented by groups of meristematic cells in which one or two vascular elements are to be discerned.

In a third leaf, no vascular tissue is differentiated at this stage (Text-fig. 3, A, L_3). The plumular axis is extremely short; there are no internodes, so that the fusion of the foliage leaves, and of the cotyledons, with the stem takes place very nearly at the same level. The leaf-traces generally continue their parallel descending course right down to the point of junction of the leaf and stem, but this is not always the case; several examples have been observed where the traces on the flanks of the petiole run tangentially in the leaf-base itself, as it joins the axis, so tending to spread themselves out to that side of the axis removed from the middle of the leaf.

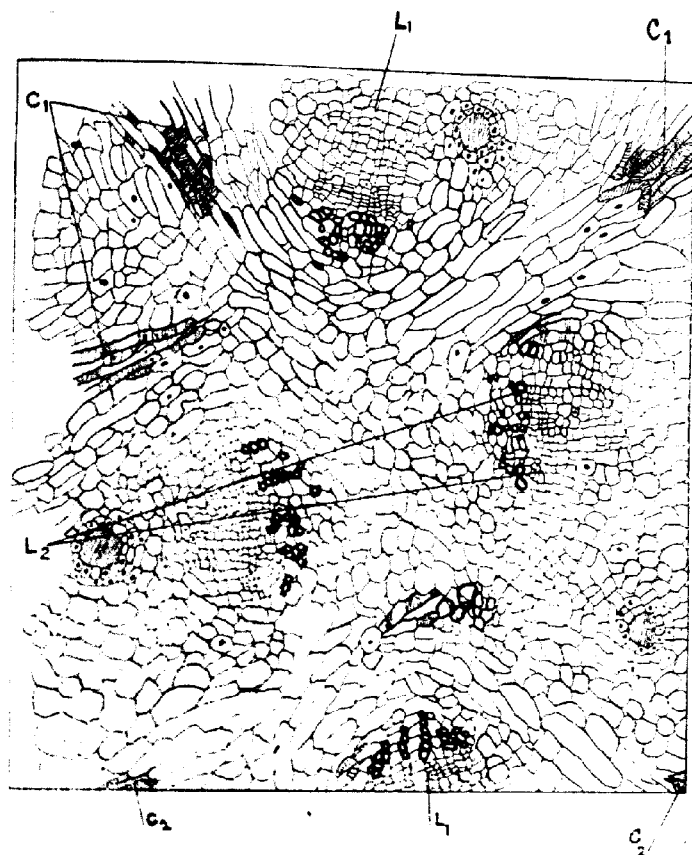


TEXT-FIG. 3. A. Transverse section of seedling above stem apex, showing cotyledon tube and traces, and first three plumular leaves, in two of which leaf-traces are developed. $\times 3$. B. Transverse section of same seedling at point where fusion of cotyledon tube and of foliage leaves with axis is nearly complete. $\times 3$. C. Transverse section of same seedling just above the cotyledonary node. $\times 8$. C_1, C_2 = traces of Cotyledons I and II; $L_1^1-L_1^4$ = traces of first plumular leaf; $L_2^1-L_2^3$ = traces of second plumular leaf; $\bullet-\bullet$ marks the intercotyledonary plane.

In the more typical case described above, there are, just above the cotyledonary node, four—or three, by the fusion of two—traces in the base of each cotyledon (Text-fig. 3, A), six in the first foliage leaf (Text-fig. 3, A, $L_1^1-L_1^6$), and nine in the second (Text-fig. 3, A, $L_2^1-L_2^9$). These all pursue a longitudinal and parallel course in the bases of their respective leaves, and the condition of affairs at the level where fusion with the axis is just commencing is shown in Text-fig. 3, A.

(f) *Course of the leaf-traces in the epicotyl.* The traces of the first foliage leaf now fuse into two bundles (Text-fig. 3, B, L_1), which eventually lie at opposite ends of a diagonal, the plane of which is removed by 90° right and left from the midrib of the leaf to which they belong, i. e. is parallel to the adaxial surface of the leaf. Between the traces of the first and the succeeding leaf there occur very irregular horizontally running anastomoses—such anastomoses may nearly encircle the stem. The centrally placed

bundles of the second foliage leaf pursue a nearly direct and longitudinal course in the axis. The marginal ones, on the other hand, pass tangentially in a nearly horizontal plane round the axis, right and left from the position of the leaf margins (Text-fig. 3, B. L_2). Fusions between individual traces occur.



TEXT-FIG. 4. Transverse section of cotyledonary node. $\times 250$. C_1, C_2 = cotyledon traces; L_1 = traces of first plumular leaf; L_2 = traces of second leaf. $\times 150$.

The final result is that these traces are also reduced to two bundles (Text-fig. 3, C. L_1), of which one, formed by fusion of the midrib system of traces, lies in the proximal portion of the central cylinder, i.e. vertically below the midrib of the leaf to which it belongs, while the second, formed by fusion of marginal traces, lies in the distal portion of the

cylinder, i. e. removed by 180° from the position of the leaf midrib (compare Text-fig. 3, C).

We see, therefore, that there are no 'direct' and no 'girdling' traces for the first foliage leaf, but that all pursue a more or less oblique course, and that the most oblique strand of this leaf encircles the axis for 90° , or rather less. The second leaf, on the other hand, has both 'direct' and 'girdling' traces—i. e. traces which encircle the axis for 180° . In this leaf, then, the typical Cycadean vascular supply is established.

We have thus established, at the cotyledonary node, the vascular arrangement figured in Text-fig. 3, C. The cotyledonary traces are grouped diagonally; there are three traces in each cotyledon, and they are arranged in groups of two traces and of one respectively, opposite to the primary medullary rays. They now pass rapidly inwards (Text-fig. 4) through these medullary rays; the xylem and phloem masses of cotyledon and leaf-traces fuse, and so an irregular central vascular cylinder is formed, which soon becomes the typical protostele of the upper region of the hypocotyl (Text-fig. 1).

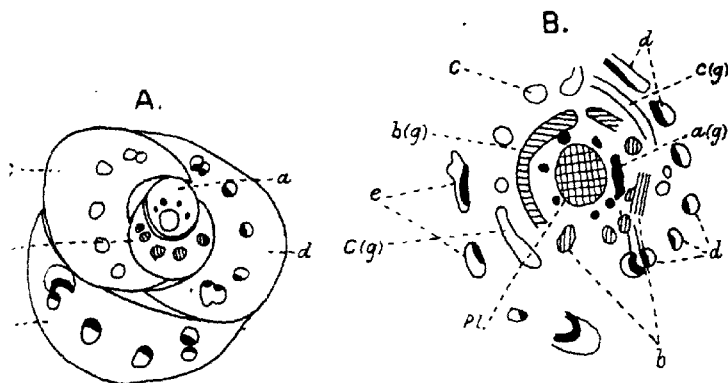
II. THE YOUNG PLANT.

A. External appearance. These plants (Plate XXII, Fig. I), which have produced some twenty leaves, have a short, tuberous stem, about 5 cm. long, clothed with persistent leaf-bases, a swollen turnip-like hypocotyledonary region, and a long, little-branched tap-root. A definite constriction occurs at the cotyledonary node. The expanded foliage leaves, about six in number, are of a varying age—the fully-formed leaf being some 80 cm. in length, and bearing between 60 and 70 pinnae. Several—generally three—rudimentary leaves are to be distinguished at the stem apex. Below the apical region are fleshy and persistent leaf-bases; these are reduced in length by the formation of successive periderm layers, until, at the base of the stem, such remains are only 1 cm. in length (Text-fig. 8).

B. Internal structure: (1) *The leaf.* In very young leaves a layer of stone cells, with lignified walls, surrounds the primordium of each future vascular strand. The fleshy base of a mature leaf is packed with starch: it is traversed by about ten endarch vascular bundles, which are arranged in an open arc. There is no extra-vascular strengthening tissue. Above the leaf-base the petiole is no longer fleshy, and contains little starch. Stone cells are found in groups in the sub-epidermal tissue. As the region of insertion of the pinnae is approached, these form an almost continuous layer—several cells thick—beneath the cuticular epidermis; in this region they are also found surrounding the vascular bundles. Numerous bundles, derived from those of the base by repeated dichotomy, and arranged on the Ω plan, traverse the petiole. These bundles are mesarch in the middle region of the petiole, becoming exarch towards its distal end. Two traces.

derived from the arms of the irregular Ω , enter each pinna; these soon dichotomize, and there are four parallel bundles throughout the greater portion of its length; near the apex, these are again reduced to two by fusion.

(2) *The stem apex and leaf-traces.* The youngest leaves are fleshy, hooded structures which overtop the stem. At the immediate apex no distinction of layers can be made out; but immediately below there is a solid, central meristematic dome, occupying the centre of the stem, from which the leaf-traces arise. The traces themselves are not distinguishable within the cylinder at this level, but are recognizable as they leave it.



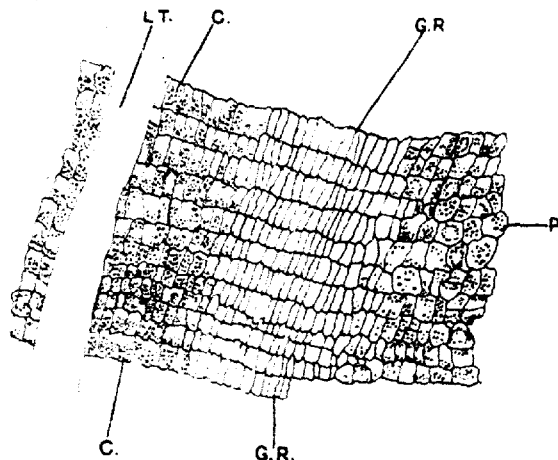
TEXT-FIG. 8. A. Transverse section of plant just above stem apex, showing bases of five youngest foliage leaves. B. Transverse section of same plant taken just below stem apex, showing vascular bundles of young foliage leaves in stem. ● = bundles of leaf a (shaded); ▨ = bundles of leaf b (hatched); ○ = bundles of leaf c (unshaded); a, b, c, d, e = first five foliage leaves in order of development; a(g) = girdling traces of leaf a; b(g) = girdling traces of leaf b, and so on; Pl. = pith. N.B. Peripheral portions of sections, showing traces of older leaves, are omitted in each case for the sake of clearness. × 3.

Each leaf receives 4-6 strands, which arise in the same horizontal plane at points widely distributed round the central cylinder (Text-fig. 5). Of these strands, those which originate in that half of the central cylinder adjacent to the leaf pursue a radial and slightly ascending course into the middle (midrib) region of that leaf, while those which arise from that portion of the cylinder which is removed by 180° from the leaf midrib swing round right and left from their point of origin, pursuing tangential and slightly ascending courses into the marginal regions of the leaf (Text-fig. 5. Cf. A and B).

The first system, which may be designated the midrib system, represents the 'direct' strands of the mature leaf. The second, marginal system constitutes the 'girdling' strands, the tangential portions of their course

being the 'girdles' of the mature structure. In the absence of internodes the courses of all these traces are nearly horizontal.

The central meristematic dome of tissue soon becomes hollow, and the development of a starchy pith is distinguishable about half a millimetre behind the apex. In this region, cell-formation is restricted to a zone of tissue on the periphery of this central dome; in this meristematic zone cell-division occurs by tangential walls only, and, as result of this method of growth, radial rows of starch-containing parenchymatous cells are added to both pith and cortex (Text-fig. 6).



TEXT-FIG. 6. Portion of longitudinal section of stem, just below stem apex, showing the 'growth ring' or procambium, and cells of pith and cortex which have been formed by it. P. = pith; G.R. = growth ring (procambium); C. = cortex; L.T. = leaf-trace, details of which are omitted. $\times 110$.

We have thus, in the primary tissues near the stem apex, a method of cell-formation which, in its restriction to a definite zone and in its cell-division in one plane only, resembles 'secondary' growth in thickness of the mature stem. This hollow cylinder—developed on the periphery of the central dome, the plerome, and ring-like in transverse section—probably represents the 'procambial ring' referred to by previous writers.^{1,2} This method of growth adds very rapidly to the girth of the stem. A considerable amount of cortical tissue is interpolated between the central dome and the tangential portions of the girdling leaf-traces, and thus these 'girdles' are carried gradually, in the course of growth, to the periphery of the stem; while maturing leaves are borne outwards rather than upwards away from the stem apex.

¹ Mettenius: Beiträge zur Anatomie der Cycadeen. Abhandl. Königl. Sachs. Gesellschaft der Wissenschaften, v, 1861, p. 573.

² Robertson: Notes on the Anatomy of *Macrozamia plicata*, loc. cit.

(3) *The vascular cylinder.* In the upper region of the stem described above, there is very little development of lignified tissue, the only xylem elements being differentiated in the leaf-traces. About $1\frac{1}{2}$ cm. below the apex, the meristematic dome of tissue, pith, and peripheral 'growth zone' is replaced by a ring of collateral endarch bundles surrounding a pith. These bundles represent the stelar portion of the traces of older leaves.

The transition from a meristematic ring of tissue, with an occasional lignified element in connexion with an entering leaf-trace, to a complete ring of endarch and secondarily thickened bundles, is a somewhat sudden one, and suggests that we are perhaps dealing with structures appertaining to two successive periods of developmental activity, separated by a quiescent period.

There is thus established a ring of collateral vascular strands, the ring thus constituted being completed by the development of a small amount of interfascicular xylem and phloem.

The wood consists of irregular rows of xylem elements, having multiserial pits on their radial walls, and separated by broad medullary rays. The phloem, composed of large- and small-celled elements arranged in radial rows, is lignified in older parts of the stem.

(4) *Cortex.* Below the level at which this ring is established there may be distinguished in the internal region of the cortex—hitherto homogeneous and packed with starch—patches of tissue which are differentiated from the rest of the cortex by the complete absence of starch. These patches, occurring just outside the stele, correspond topographically to the 'cloudy' tissue which occurred in preparations made by Marsh¹ of the stems of *Stangeria paradoxa*, and represent, perhaps, the same elements.

(5) *Tangential extension of xylem and phloem elements.* At the level of the establishment of this starch-depleted tissue in the inner cortex a change occurs in the constitution of the central vascular cylinder, to which the cambium has been adding radial rows of xylem and phloem elements in the usual way. In this region the cells abutting on the cambium are longitudinally extended; this tangential stretching, which is undoubtedly due to the elongation in a tangential direction of the cambial cells themselves, becomes greater as each succeeding element of xylem and phloem is differentiated, until a central cylinder is established which consists, on the side abutting the pith, of the usual radial rows of tracheides, but of which the peripheral xylem runs in a tangential direction round the stem (Plate XXII, Fig. 11). The width of these two xylem bands becomes approximately equal. The phloem participates equally in this peculiar method of growth, so that abutting on the cambium externally are tangentially running phloem and phloem fibres. This gradually gives place to the earlier-formed, normally-directed phloem, and then to the lignified fibres which form the

¹ Marsh: loc. cit.

outer boundary of the vascular ring. This structure suggests in some of its features that found by Dr. Stopes¹ in the stem of the cretaceous fossil *Colymbites Edwardsii*. The wood of *Colymbites* is a single vascular cylinder, composed of alternating bands of radial and tangential xylem elements, and it seems likely that this whole structure is the product of one cambium. It is of interest to find, in a living Cycad, a vascular cylinder which shows a similar alternation of a radial and a tangentially elongated band of wood, and which is therefore in some degree comparable with that remarkable fossil.

In the living plant this arrangement extends to the phloem also, which was not preserved in the fossil (Plate XXII, Fig. III).

It is unfortunate that these plants were fixed very roughly, and that the cambial tissue is somewhat collapsed in all specimens. Nevertheless, it seems quite certain that the cambium cells were themselves tangentially extended, and, indeed, the whole phenomenon is probably the result of this stretching of the cambium. The causes which may have led to this will be discussed later.

(6) *The anomalous ring.* Soon after the establishment of tangentially running elements in the xylem and phloem of the vascular cylinder, patches of meristematic cells are observed in the starchy tissue which lies between the peripheral lignified fibres of the vascular cylinder and the inner cortical region of starch-depleted tissue referred to above. The initiation of this secondary cambium seems to bear some relation to the stretching phenomenon just described. Tangentially running elements do not appear simultaneously at all points of the stem; there may be a considerable development of those elongated tracheides at one or two points of the circle, whereas at other points normal radial elements are still being formed. In such cases the abnormal cambium seems to arise first outside those areas of the vascular cylinder which have undergone tangential extension. It is suggested that the two phenomena may be due to some common cause. Within this abnormal cambium, and probably formed by it, groups of extremely irregular lignified 'transfusion' elements are observed, recalling the tracheides found on the internal faces of anomalous rings in the mature stem of this species.² In that region of the stem where the first anomalous cambium is well established and is becoming functional, there are very obvious patches of conducting elements, forming an integral part of the anomalous ring (Plate XXII, Fig. III), but which would appear to be developed in connexion with the outgoing leaf-traces. At this stage the strand itself cannot usually be followed far into the cortex, and may often seem to end in the anomalous ring, thus giving rise to the appearance of special 'anastomoses' between the two systems. Whether anastomoses may occur

¹ Stopes: Catalogue of the Cretaceous Flora. Part II, p. 314 et seq.

² Worsdell: loc. cit.

apart from the leaf-traces has not been determined. The cambium of these 'anastomoses' becomes continuous with the 'anomalous' cambium, and thus a complete cambial ring is eventually established, appertaining to a vascular cylinder of extreme irregularity lying outside the normal vascular ring (Plate XXII, Fig. III).

(7) *Tangential extension in the anomalous ring.* The second ring, once well established, is seen to become affected by the phenomenon of tangential stretching already described. At the extreme base of the stem we have thus the following structure (Plate XXII, Fig. III):

A pith, surrounded by a vascular cylinder composed of radially arranged wood, tangentially running wood, tangentially elongated cambium, tangential phloem, radial phloem. Outside this, a second vascular ring which, though more irregular and less complete than the normal ring, is composed of the same tissues arranged in the same order.

(8) *The cotyledonary node.* At the cotyledonary node the complication becomes extreme. Seen both in longitudinal and transverse section, the xylem tracheides pursue courses of extraordinary irregularity, often doubling back on their original path. In this region the two vascular cylinders become very closely approximated, and it is sometimes difficult to say to which cylinder certain elements belong; there are indications that a third anomalous ring is in process of formation, but the whole structure is too irregular to admit of any very satisfactory interpretation (Plate XXII, Fig. IV).

(9) *The root.* The extremely long tap-root is usually diarch in structure, and no increase in the number of poles, as the apex is approached, such as has been described for seedlings by Hill and de Fraine,¹ has ever been observed. In one case, a triarch root, reducing to diarch, occurred. The structure is of the normal gymnospermous type; the many-layered pericycle consists of cells tangentially elongated and flattened, many of these having lignified walls. In no case has any anomalous thickening been detected (cf. Gregg²).

As the hypocotyledonary region is approached there is evidence that there is stretching, and finally division, of the cells of the usually uniseriate medullary rays, until these rays are 4-5 cells wide.

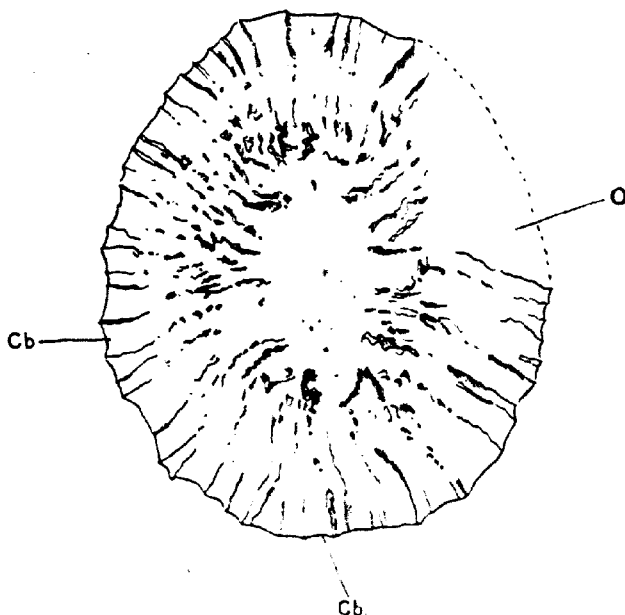
(10) *The swollen hypocotyl.* The swelling characteristic of the upper hypocotyledonary portion of the axis is due to an enormous increase, as compared with the root region, in the parenchyma present. Pith and pericycle are both considerably enlarged by the stretching and division of their cells; the xylem is invaded by parenchyma; the medullary rays increase in size, while each radial row of wood elements becomes broken up into several groups by the intrusion of non-lignified tissue (Text-fig. 7).

¹ Hill and de Fraine: loc. cit.

² Gregg: Anomalous Thickening in the Roots of *Cycas siamensis*. Ann. Bot., vol. i, 1887.

There is some breaking down of the pith cells, probably due to mucilagization of their walls; these collapsed cells give a 'stringy' appearance to both pith and pericycle, very characteristic of this portion of the plant.

The stretching and ultimate collapse of these cells of the pith affect the xylem tracheides abutting on this region, which become bent and doubled up in all directions, producing an effect of extraordinary distortion in sections of the plant at this level. The only portion of the conducting system of the root which does not become involved in this distortion is



TEXT FIG. 7. Xylem and cambium of hypocotyl, as seen in transverse section. Radial lines indicate radially arranged rows of xylem elements. Hatchings indicate xylem elements running obliquely or longitudinally in the plane of the section. At O the section is incomplete. Cb = cambium. $\times 3$.

the zone containing the cambium and those tracheides to which it has recently given rise.

When this great distension of the hypocotyl takes place, all mechanical tissue tends to disappear. The wood, as we have seen, is widely separated by parenchyma; the fibres of the phloem cannot be identified, while the lignified cells of the pericycle are also lost. Such is the structure beneath the cotyledonary node.

SUMMARY OF RESULTS.

We can distinguish, in the axis of such a plant, the following regions:

1. An apical region, where new leaves are formed and gradual growth in length occurs (Text-fig. 8, A).

2. A sub-apical region, where growth in girth is actively occurring. This is brought about by a specialized peripheral layer of the central plerome, which, dividing only by tangential walls, adds parenchymatous tissue to pith and cortex in a manner reminiscent of the formation of secondary tissues by a cambium (Text-fig. 8, B).

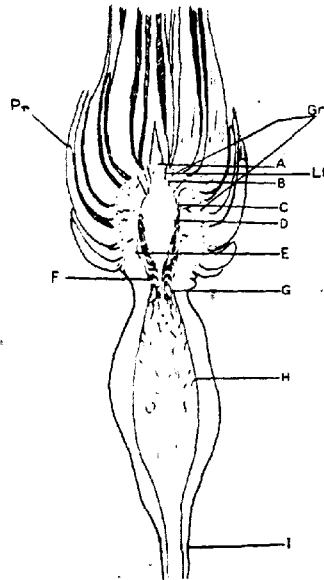
3. Below this is established a collateral vascular cylinder, in which the xylem is largely secondary. In this region the cortex is differentiated into two regions—an inner 'cloudy' tissue and an outer starchy parenchyma (Text-fig. 8, C).

4. The cambium of the vascular cylinder is tangentially extended, and xylem and phloem elements which now arise partake of this elongation. In this region the beginnings of the cambium of the anomalous ring are first distinguished (Text-fig. 8, D; also Plate XXII, Fig. II).

5. Irregular transfusion tracheides are formed by the anomalous cambium, and the connexions or 'anastomoses' are being established by the normal cylinder. A complete although irregular ring is fully established—the first anomalous ring—and the cambium of this ring forms xylem and phloem in the usual way (Text-Fig. 8, E).

6. The phenomenon of tangential stretching makes its appearance in the anomalous ring. (Text-fig. 8, F, also Plate XXII, Fig. III).

7. The cotyledonary node is a region of extreme complexity, and marked by distortion of the tissues (Text-fig. 8, G; also Plate XXII, Fig. IV). This may be in some measure due to the fact that there exists at the node a definite constriction of the plant axis; further, that it abuts on—



TEXT-FIG. 8. Young plant cut longitudinally nearly in the median plane, and treated with phloroglucin to bring out xylem. For lettering A-I see text. $\times \frac{1}{2}$. Pr. = periderm; Gr. = girdling leaf-trace; Lt. = longitudinal leaf-trace.

8. The swollen hypocotyledonary region of the root, in which all the earlier-formed xylem has become disrupted and doubled up, as the result of the extreme distension of this region. In view of this distortion of the tissues, it seems probable that conduction is maintained only via the newly-formed secondary elements which border the whole conducting cylinder (Text-fig. 8, H).

9. An extremely long root of normal diarch structure, which develops few branches at this stage; this is secondarily thickened in the normal way (Text-fig. 8, I).

III. THEORETICAL CONSIDERATIONS.

(i) *The Anomalies of the Parenchymatous System and the Phenomenon of 'Girdling'.*

The form of vascular supply which characterizes the mature foliage leaf would seem to be the result of two factors. In the young leaf a broad decurrent base of insertion necessitates the derivation of the vascular supply from points widely separated on the vascular cylinder. The extreme telescoping of the axis, and the complete absence of internodes, has as a result that those strands of the leaf-trace which are derived from regions of the vascular axis removed by nearly 180° from the leaf midrib pursue tangential and nearly horizontal courses into the leaf margins.

This condition, established near the stem apex, is subsequently modified by the activity of the specialized 'growth layer' or cambium, which occurs in the sub-apical region. The activity of this layer results in the interpolation of a considerable amount of parenchymatous tissue between the point of origin of the lateral leaf-trace and the tangential portion of its course. As a result of this, the tangential portion—'girdle'—of the trace is carried towards the periphery of the stem, while its radial connexion with the stele becomes longer. The leaf-supply characteristic of the mature leaf is thus established. The structure of the leaf-traces seems to harmonize with this view. They are composed of open, scalariform vessels, as opposed to the multiseriate pitted vessels of the stem, and this retention of a primitive feature lost in the adult stem may be interpreted as a device for keeping such traces elastic as the girth of the stem increases (cf. Worsdell¹).

It has, further, been shown that the earlier-formed wood vessels of these leaf-traces are born apart in the mature condition. This is probably best interpreted as a result of the stretching strain imposed on their tissues. If this view is the correct one, the 'girdle' is no new phenomenon whose origin is 'a very obscure problem'.² Nor is it true that 'traces of the youngest leaves at first ascend in an almost perpendicular direction, but,

¹ Worsdell: loc. cit.

² Coulter and Chamberlain: *Morphology of Gymnosperms*, p. 103.

'during further growth, gradually assume an almost horizontal position' (Mettenius¹).

We have here the normal leaf-supply characteristic of a large leaf with broad base. The nearly horizontal course of all the strands of the trace is due to the telescoping of the axis; in the case of the marginal traces, this course is necessarily tangential also, on account of the origin of these strands from the distal half of the axis, associated with the broad leaf insertion. The removal of these 'girdles' to the periphery of the stem is due to the subsequent increase in parenchymatous tissue of pith and cortex, as also is the virtual intercalation of radial portions near their insertion on the axis.

(ii) *The Anomalies of the Vascular System.*

The anomalous rings. The result of this investigation harmonizes two divergent views. Some workers have derived the anomalous rings from a pericyclic cambium,² others from branches given off from the normal ring.³ It is seen that, in *Macrozamia Fraseri*, both factors play a part in their development.

It is established that, in the plants under examination, the appearance of the anomalous ring, at any point in the stem, is preceded by two occurrences which have possibly a physiological significance.

(1) Starch depletion of the inner cortical tissues.

(2) Tangential extension of the tracheides and phloem elements being formed by the cambium of the normal cylinder. This tangential elongation is probably the result of that definite horizontal growth established near the stem apex,³ while the horizontal growth is itself the result of those environmental conditions which have imposed slow growth and the tuberous geophilous habit on these plants. The task of supporting large leaves on a very shortened axis would seem to make a broadening of this axis intelligible. This adaptation would bring other difficulties in its train; one result already attributed to it is the complication in the course of the strands entering the mature leaf. Another result would probably be strain on the established tissue beneath the apical zone, and a consequent stretching of the non-lignified elements along the line of least resistance. To some such sequence of events the gradual extension of the cambial elements and the tissues derived from them is probably due. That this would not react favourably on conduction seems evident, and the starch-depleted layer developed at this point in the cortex possibly reflects a functional starvation of that tissue, whether due to this distortion or to the general inadequacy

¹ Mettenius: loc. cit.

² Constantin and Mohr: Sur l'origine des faisceaux libéro-ligneux surnuméraires dans la tige des Cycadées. Bull. Soc. Bot. France, xxxii. 173, 1885.

³ Matte: loc. cit.

of the conducting system. It may be that the need of a greater development of conducting tissue thus indicated is met by the plant by the formation of the anomalous ring-structures described.

The whole habit of the plant, with its tuberous hypocotyl, in which early-formed xylem is doubled up in an extremely complicated manner, the broad and fleshy stem, with its great and definite parenchymatous development, obviously differs so much from the normally elongated dicotyledonous and gymnospermous plants that it is tempting to suppose that habit and anomalous vascular structure are not merely correlated, but that the former is causally related to the latter. If this be so, we are dealing here with phenomena which may be compared, in a general way, with the anomalous rings of the beetroot and the anomalies found in such plants as *Welwitschia* and *Isoetes*, and the existence of these would seem not in itself to carry with it any phylogenetic significance.

The cotyledonary node. This is simple in structure in the seedling; in the young plant the same fundamental structure obtains (Plate XXII, Fig. IV), but it is obscured by the extreme complication of the secondary tissues. As this complication is accompanied by very great distortion in tissues both of epicotyl and hypocotyl, it seems unlikely that any such phylogenetic importance as has been attributed to it¹ can be attached to the structure at this stage.

No *concentric* bundles have been observed in the course of this investigation. Many horseshoe-shaped ones have been seen; these were always found to be transient agglomerations of the usual Cycadean endarch or mesarch traces.

Finally, I should like to express my sincere thanks to Dr. Thomas, who suggested this work, for her very helpful advice and criticism while it was in progress. Further, to thank Miss A. J. Davey for help in the preparation of sections, &c., and my friend Miss Prankerd for her sympathetic encouragement.

¹ Worsdell: loc. cit

· EXPLANATION OF PLATE XXII.

Illustrating Miss E. J. Hatfield's paper on the Anatomy of the Seedling and Young Plant of *Macrozamia Fraseri*.

The photographs are by Mr. Pittock.

Fig. I. Young plant with foliage leaves cut off just above stem apex. C.N. = cotyledonary node; Hy.C. = swollen hypocotyl. \times about 1.

Fig. II. Transverse section of stele of young plant (at level D of Text-fig. 8), showing radial and tangentially extended xylem of normal vascular ring. R.X₁ = radially arranged secondary xylem; T.X₁ = tangentially extended secondary xylem; An. = 'anastomoses'. \times about 5.

Fig. III. Transverse section of central portion of young stem (at level F of Text-fig. 8), showing xylem of 'normal' and first 'anomalous' vascular rings. The xylem of both rings and the phloem fibres of the normal ring exhibit the phenomenon of tangential elongation. R.X₁ = radially arranged xylem of 'normal' ring; T.X₁ = tangentially elongated xylem of 'normal' ring; T.Ph. = tangentially elongated phloem fibres of 'normal' ring; R.X₂ = radially arranged xylem of first 'anomalous' ring; T.X₂ = tangentially extended xylem of first 'anomalous' ring; An. = 'anastomoses' connected with outgoing leaf-traces. \times about 3.

Fig. IV. Transverse section of inner portion of plant axis at cotyledonary node. (Compare with Text-fig. 4.) Shows three, or perhaps four, xylem rings, and these all exhibit the phenomenon of tangential extension. Lt. = xylem elements of outgoing leaf-traces. \times about 3.

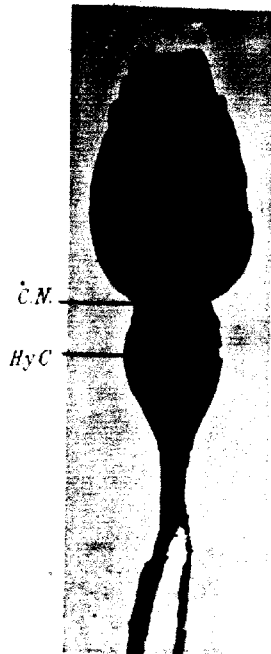


Fig. 1.

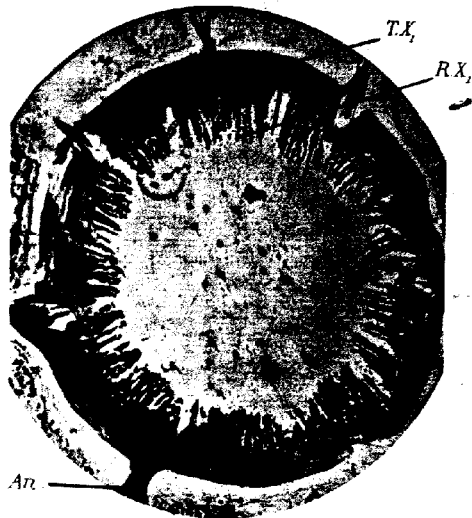


Fig. 2.



Fig. 3.



Fig. 4.

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On the Behaviour during Drought of Leaves of two Cape Species of *Passerina*, with some Notes on their Anatomy.

BY

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With thirteen Figures in the Text.

ONE of the features of the vegetation of the Cape Peninsula which immediately strikes the observer is the prevalence of shrubby plants with small, often narrow leaves. Closer observation shows that many of these have leaves of a strictly ericoid type, with grooves usually more or less filled with hairs, the stomata being confined to the epidermis lining the groove. These plants belong to various families. Of the numerous species of *Erica* and other genera of Ericaceae, all but a few possess leaves of this type. They are found also in *Stilbe ericoides* and *S. vestita* (Verbenaceae), *Phytica* spp. (Rhamnaceae), *Rhus rosmarinifolia* (Anacardiaceae), *Passerina filiformis* and *Chymococca empetroides* (Thymelaeaceae), *Grubbia rosmarinifolia* (Grubbiaceae), and in several genera of Compositae, including *Stoebe*, *Disparago*, *Elytropappus*, and *Metalsia*. This list is by no means exhaustive.

A fact of some interest with regard to a number of the species was communicated to the South African Association for the Advancement of Science at the meeting in Bulawayo in July, 1920, namely, that the grooves vary in the width of their opening with changes in the conditions. In the dry summer of 1920, in February and March, the leaves of *Erica* spp., *Stilbe vestita*, and *Passerina filiformis* were found with the grooves quite or nearly closed on plants in specially dry situations.

In *Passerina* the closure of the grooves was observed more frequently than in the other species. This was therefore selected first for detailed study.

Passerina filiformis is widely distributed in South Africa. In the Cape Peninsula it is very common, extending from a little above sea-level to at least a thousand feet on the mountain slopes.¹

¹ Bolus and Wille-Dod: Flowering Plants and Ferns of the Cape Peninsula. Trans. S. African Phil. Soc., xiv, 1903, p. 313.

[Annals of Botany, Vol. XXXV. No. CXL. October, 1921.]

Observations in the field, made subsequently to much of the experimental work described in this paper, make it clear that two forms are to be distinguished among the plants that had been used, one of which is almost certainly *Passerina filiformis*, Linn.; the other resembles *P. falcifolia*, C. H. Wright, though it does not agree in all respects with Wright's description in the 'Flora Capensis'.¹

The former commonly reaches a height of four or five feet. It has numerous erect branches and more or less adpressed leaves, decussately arranged and, when young, closely adpressed to one another in four conspicuous ranks.

The other form has a more spreading, bushy habit; the leaves are longer, and are not adpressed except when young, but spread at an angle of 45° or more from the stem.

In much-shaded situations, plants belonging to the latter form are found with a very lax habit, with leaves spreading still more widely and branches slender and straggling.

In both forms the leaf is grooved on the upper side, the groove being thickly lined with close, woolly hairs. When the leaves are adpressed the groove is brought near to the surface of the stem, which is pubescent when young.

In herbarium material the leaf-grooves are tightly closed so that the leaves appear acicular.

Closure of the Groove and Water Content.

In the dry season (from January to March) the grooves are narrowed by the approximation of the two sides of the leaves, until the edges may meet over the groove and completely enclose it. Closure proceeds from the tip towards the base, where communication with the outer air is maintained except in extreme drought.

The following experiment shows how closure is correlated with diminishing water content:

A shoot was brought to the laboratory in the latter part of February, 1920, and kept in a full vasculum, weighed the following day, and then put with the cut end of the stem in water under a bell-jar. Most of the leaves had their grooves tightly closed. By the next day they had opened well. After another day the shoot was finally weighed and its dry weight determined. The results are given in Table I.

¹ *P. falcifolia* is described as having the bracts densely woolly within. In my specimens there is only a narrow band of woolly hairs, continuous with the groove of the narrow leaf-like apex, while the wings are glabrous. No record of *P. falcifolia* from the Cape Peninsula is mentioned in Flora Capensis, and Bolus and Wolley-Dod (loc. cit.) did not separate the form in question from *P. filiformis*. The leaf characters, however, are more like those of *P. falcifolia* and distinguish it from all the other species described.

TABLE I.

	Weight in grm.	Water Content.	
		% of fresh weight.	% of dry weight.
Original weight, grooves closed	6.81	28.6	40
After 6 hours, grooves mostly still closed	7.50	35.0	54
After 22 hours, grooves open	9.05	46.2	86
After 48 hours, grooves open	9.23	47.2	89
Dry weight	4.87	—	—

The water content of the shoot as gathered was thus surprisingly low. The experiment showed nevertheless that the shoot was fully able to recover its turgor and the leaves to open their grooves. Even the maximum water content of the turgid shoot was no more than 47.2 per cent.

Range of Water Content in the Dry Season.

Following on this experiment the fresh weights were determined of a large number of shoots, with precautions against loss of moisture in the interval between collecting and weighing (March 6, 1920); the condition of the leaves was noted and their dry weights found. The shoots were taken from bushes in a variety of situations, probably also of both forms, which at the time I had not realized as specific. In the driest places many of the plants were yellowish or brownish in colour, the insolated sides of twigs and even of individual leaves contrasting markedly with the opposite sides, which were still more or less green.

The shoots were separated into three groups according as the leaf-grooves were open, closed, or open in older leaves and closed in younger except at the base. The range of water content in each group is given in the following table:

TABLE II.

	Range of Water Content.	
	% of fresh weight.	% of dry weight.
Grooves open	45.3-37.1	83-59
Some grooves open, some closed	34.5-33.3	53-50
Grooves closed	34.1-25.7	52-35

These figures and those in Table I show that at this season of the year the critical water content at which the grooves closed completely was about 34 per cent. The water content could, however, sink farther to 26 per cent. (about a third of the dry weight) apparently without permanent injury resulting.

This very low water content in still living vegetative shoots appears to be without recorded parallel.

Cases are of course known of plants which become practically air-dry without injury. A very interesting example of such a plant (*Myrothamnus flabellifolia*) grows in cracks on exposed granite slopes in Rhodesia. In the dry season the leaves become plicately folded and tightly adpressed to one another at the tips of the twigs, while these in their turn curve inwards. When the rains come, leaves and branches expand again.

In July, 1920, I collected small twigs of this plant on the Matoppo Hills, near Bulawayo, in the dry folded condition, enclosed them in a corked tube, and on my return to Cape Town a fortnight later found the water content to be only about 7 per cent. Such plants as this must be completely air-dry and dormant.

Passerina, on the other hand, still transpires slowly and is not dormant, however sluggish its vitality may become under such extreme conditions. Determinations of the water content of other plants show that, although the minimum is so far lowest in the case of *Passerina*, the shoots of not a few sclerophyllous plants may have a water content falling considerably

below 50 per cent. in the summer.

As might be expected, a higher percentage of water is found at other seasons of the year. Shoots of *Passerina* collected in mid-September, 1920, had a water content of 59.61 per cent. Even this figure is lower than might have been anticipated in shoots which were collected after rainy weather, towards the end of a wet winter, early

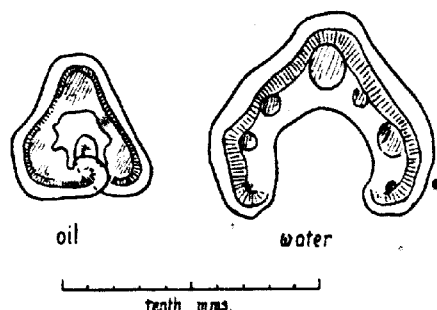


FIG. 1. Tracings of adjacent sections from a leaf of *Passerina* cf. *falcifolia* in which the groove was tightly closed; left, in oil, the edges overlapping; right, in water, groove open, tissues expanded.

on a cloudy morning. After the first week of summer weather, with a fairly strong dry south-east wind and brilliant sunshine, the water content of shoots of both species, collected in the afternoon, was 56 per cent.

Mechanism of Closure.

When a transverse section of a closed leaf is put into water, it expands at once and the groove opens widely. In order to estimate the degree of expansion involved, adjacent sections cut dry were mounted, one in oil, the other in water, and camera lucida outlines compared.

Cases were often observed of thin sections, either dry or mounted in oil, having their edges overlapping, showing that in the intact leaf the edges were pressed together under tension (Fig. 1).

On comparing the sections the change of dimensions proved very considerable. It was most apparent in the mesophyll, where in the closed condition the palisade tissue was so condensed that the individual cells were often difficult to distinguish, while the spongy tissue was relatively still more contracted in volume. The cells of the outer epidermis were also contracted in height.

The outer epidermis is provided with a very thick cuticle. The inrolling or opening of the leaf is therefore to be attributed to changes of volume taking place within this resistant cuticle. But it is a question of some importance whether changes of turgor are wholly responsible, or, if not, what other forces bring about the movement.

Figs. 2 and 3 illustrate the chief structural details involved. The two sections represented in Fig. 2 are from old healthy leaves from (a) an ordinary sturdy plant of *Passerina filiformis* with more or less adpressed leaves, (b) an extreme shade plant of *Passerina* sp. cf. *falcifolia*, with widely spreading leaves. The two are in most respects very similar. The most conspicuous difference is in the main bundles, the fibres in (a) interrupting the palisade tissue and reaching the epidermis. This difference appears to be specific; the continuous palisade of (b) is characteristic of the form with

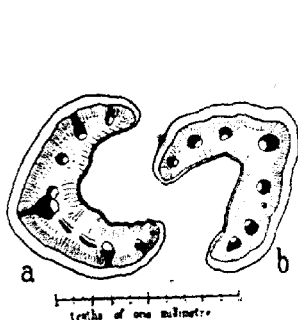


FIG. 2. Diagrams of sections of old healthy leaves of (a) *P. filiformis*, (b) extreme shade plant of *P. cf. falcifolia*. Fibres black; arrangement of mesophyll cells indicated by the shading.

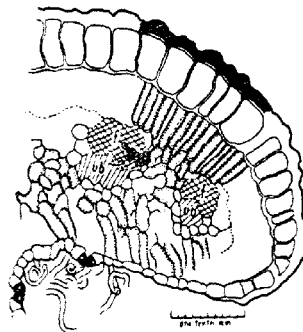


FIG. 3. Part of section of leaf of *P. cf. falcifolia*. Cuticle of outer epidermis strongly shaded, also the raised guard cells; f, fibres; vb, vascular bundles.

spreading habit. It is shown also in Fig. 3, which is from a plant of this form growing in the open.

In this figure the structure is shown more in detail. The outer epidermis is of relatively large cells, deeper than wide. The whole of the shaded part of the outer wall is strongly cutinized. The inner epidermis is thin, clothed with curly hairs, and has numerous raised stomata.

To return to the question of mechanism; experiments were made with sections transferred from water to 10 per cent. sodium chloride solution (causing plasmolysis), chloroform water, formalin, and other killing reagents. Plasmolysis was accompanied by only a small narrowing of the groove. Killing with formalin or chromacetic gave a negative or very slight result. With chloroform water the result varied, and this was traced to the direct effect of absorption of chloroform by the cuticle. A section

mounted on a cover-slip over a cell containing chloroform rapidly closed, but opened again when remounted in fresh water practically as widely as before. Careful measurement showed that the surface of the cuticle increased in length over chloroform vapour. In the particular section measured the increase was 2.3 per cent. Comparison of tracings from a section of a closed leaf, (a) in absolute alcohol and (b) after opening in water, showed no appreciable change in circumference. With chloroform, then, an increase in circumference is responsible for closure; whereas normally closure is due to the contraction of the tissues within the cuticle.

These experiments indicate that the normal position of the cuticle and water-imbibed cell-walls corresponds to the open groove, and that but little

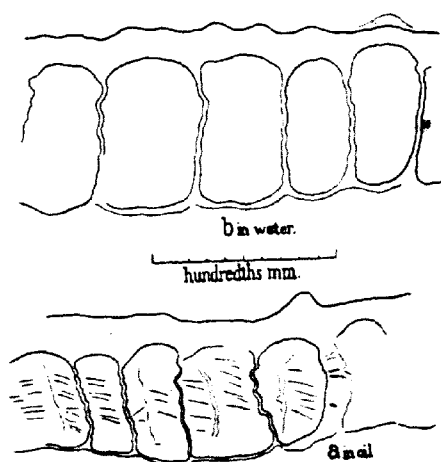


FIG. 4. Epidermis of corresponding parts of two adjacent sections; (a) in oil, showing bellows-like foldings; (b) expanded in water.

further opening results from turgor. Any increased opening in short spells of moist weather due to osmotic expansion of the living cells can be of little account. On the other hand, in sections killed in alcohol the grooves open in water. Closure must therefore be due to imbibition or cohesion forces or a combination of both.

In considering the exact location of these forces the thin inner epidermis and loose spongy mesophyll may at once be dismissed; so, too, may

the bundles (but see below as regards the fibres in *P. filiformis*, p. 597). The palisade tissue and outer epidermis both contract radially. It is, however, a tangential force which is required, and this, by its structure, the palisade parenchyma is hardly adapted to develop. Moreover, in extreme cases the palisade cells have the appearance of being pressed closely together. The epidermis alone has the tangential continuity necessary to bend the resistant cuticle by its contraction.

Cohesion. That cohesion forces are developed in the outer epidermis of the closing leaf is shown by the lateral folding of the walls. Figs. 4 a and b are from camera lucida drawings of cells from corresponding parts of adjacent sections cut dry, one mounted in oil, the other in water. The

bellows-like folding of the walls is clearly shown in *a*; in *b* the cells have increased considerably in volume and the walls are nearly straightened. This feature is characteristic of certain kinds of water-storing tissue, a point which will be considered again later. In the present connexion it is a clear indication of cohesion tension drawing the walls together, a tension which must act tangentially as well as at right angles to the surface.

As the epidermis is curved in the expanded condition, the approximation of the short inner to the longer outer walls during the contraction, which is facilitated by the folding of the lateral walls, will in itself bring about an increase in curvature. This effect will be more pronounced where the initial curvature is greater, and in fact it is in such regions that the increase of curvature chiefly occurs (cf. Fig. 1). Cohesion therefore acts in two ways to close the groove, by drawing the lateral walls together and by drawing the inner walls to the outer.

Subsidiary factors. The sap of the epidermal cells contains a substance in solution which forms in absolute alcohol a gelatinous mass slightly contracted away from the cell-wall. As to the chemical nature of the substance I have not yet been able to obtain satisfactory evidence. So long as the sap is fluid or semi-fluid it can hardly assist closure, though it may help the cells to resist the loss of water, and may also account in part for the rapid expansion of sections transferred from absolute alcohol to water. In dead cells, such as often occur in small patches in the outer epidermis of old leaves, the contents are brown and the outer wall depressed inwards. The contraction of the lumen is, however, only partial, and it seems probable that complete collapse is prevented by the gelatinous contents. A corresponding phenomenon in cells with mucilaginous inner walls is described below (p. 599).

Associated with the development of cohesion tension in the epidermal cells there will also be a contraction of the inner cellulose wall, which must lose some of its imbibed water in the maintenance of equilibrium between the imbibition and cohesion forces. This contraction would tend to bring about closure of the groove. In the expanded condition these walls bulge inwards somewhat into the palisade tissue; in the contracted condition they are sometimes nearly straight. This straightening, partly due no doubt to the contraction of the walls themselves, partly to the cohesive pull of the contracting contents, involves some deformation of the palisade layer. Any resistance offered to this deformation would assist closure. On the other hand, I have seen cases in which the inner walls were straighter in the expanded condition.

On the whole it seems clear that cohesive forces acting in the outer epidermis play the principal part in the closing mechanism. Other factors may also be concerned in variable degree, but their effects are subsidiary.

Water Storage, or Absorbing Power?

Although folding of the walls is easily observed in the epidermal cells it is not confined to them. The cells of the palisade parenchyma in tightly closed leaves may also show fine foldings which are less easily demonstrated. Fig. 5 is from a microtome section of a closed leaf, fixed in absolute alcohol and carefully embedded in paraffin.

Solereder¹ mentions the common occurrence of similar foldings in the palisade parenchyma in herbarium material of various plants, and infers that this tissue has water storage as a subsidiary function. To adopt such a suggestion in the present instance would, however, be to overlook the real significance of the phenomenon; for the amount of water that can be stored, even in the epidermis, is small. Moreover, the contracted condition may of necessity persist for a considerable time. True water-storage tissue

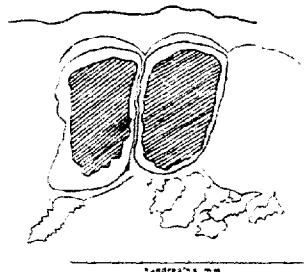


FIG. 5. Outer epidermal and palisade cells from microtome sections of closed leaf, fixed in absolute alcohol and embedded in paraffin. The contents of each epidermal cell (shaded) form a somewhat contracted brown gelatinous mass. Palisade cells with finely-folded walls.

may act either as a reservoir of water on which the active tissues of the organ itself may draw during temporary shortage, so that they may remain actively functioning, or as a reservoir which may be tapped by younger parts of the plant, the older parts yielding up their water and dying. Among succulents both alternatives are represented, often in the same plant. In *Passerina* the former alternative may apply to a limited extent, in so far as the epidermis yields water to the assimilating tissues. It can, however, only be of service in relation to temporary drought.

As regards the assimilating tissues themselves, the folding of the walls is an accommodation to a greatly diminished water content which may persist sometimes for many weeks. As the cell sap shrinks in volume the cell contracts in length rather than in breadth: it may perhaps be due in part to this regular and structurally determined mode of contraction that the cell survives. The principal fact is, however, the great diminution in volume, to which the cell-wall offers little resistance. There is no hydrostatic pressure in the contracted cell, but the full osmotic pressure of the sap is brought into play for the absorption and retention of water.²

The difficulty of detecting the foldings in the palisade cells has prevented extensive comparative observations of the behaviour in this respect of the epidermis and palisade layer. In spring, at any rate, folding

¹ *Systematic Anatomy of Dicotyledons*, vol. ii, p. 1088.

² Cf. Thoday, *New Phyt.*, xvii, 1918, pp. 108-113.

appears to begin much sooner in the epidermis. In leaves of *P. cf. falcifolia*, after partial drying in the laboratory, the epidermis showed beautifully regular foldings, while no folding could be detected in the palisade cells. On the other hand, Fig. 5 illustrates a case in which the folding was more marked in the palisade cells; this was summer material. Differences of age may perhaps be concerned here, e.g. the walls may be more flexible and extensible in young palisade cells.

However this may be, a considerable change of volume is apparent also in the palisade tissue, even when the epidermis alone shows well-marked folding.

The most reliable data are those obtained with sections mounted in absolute alcohol and afterwards transferred to water, as thinner sections can be cut with a razor moistened with absolute alcohol and the optical conditions are more favourable. In the case of a widely opening leaf of *P. cf. falcifolia* the following were the averages of numerous radial measurements of a section treated in this way:

	In Water.	In Absolute Alcohol.	Difference.
	μ .	μ .	%.
Epidermis, outer wall	23	23	—
Epidermis, lumen and inner wall	72	46	36
Palisade layer	52	36	32
Spongy tissue	197	107	46

Here, of course, the expansion is that of the tissue killed in absolute alcohol, and does not necessarily correspond with what would have occurred had they been still living. Rough measurement of sections measured dry and in water, and comparison of adjacent sections in oil and water, supported these data in a general way; but in some cases the expansion of the palisade tissue of dry sections was proportionately greater than that of the epidermis.

In view of these observations. I am inclined to regard water storage as a function of subsidiary importance even in the epidermis. The properties of the tissues will allow of some expansion during the night, when transpiration is minimal. But it is the increased power of holding and absorbing water which is of prime importance; for cut shoots, even in the laboratory in shade, when deprived of any water-supply, become nearly air-dry in two or three days in the dry season.

Significance of the large-celled Outer Epidermis.

If water storage is only of subsidiary importance, the question of the significance of the thickness of the outer epidermis still remains. Relative to the size of the leaf the cells are very large. They are also large compared with those of many larger leaves. The following table gives the height of the epidermal cells in a few instances, and in some cases the thickness of the outer wall:

Plant.	Epidermis. μ.	Outer Wall. μ.
<i>Passerina filiformis</i> }	60-95	Up to 23
" <i>cf. falcifolia</i> }		
<i>Stilbe vestita</i>	60-100	—
<i>Protea lepidocarpodendron</i>	80	30
<i>Lobostemon</i> sp.	30	—
<i>Ivy</i>	20	9
<i>Helianthus</i> (? <i>annuus</i>)	10-20	—

Stilbe vestita, like *Passerina*, has ericoid leaves. *Protea lepidocarpodendron* has large, thick and tough, lanceolate leaves. *Lobostemon* (Boraginaceae) has thick and rather fleshy, coarsely hairy leaves. All these are evergreen plants of the Cape Peninsula maquis. In the first three the thickness of the epidermis is of the same order. They may all be described as sclerophyllous, the leaves being tough. The toughness is largely due to the thick outer wall and cuticle, so that thick outer wall and thick epidermis are correlated in these instances. The data are too few, however, for generalization, and show the need for extensive comparative observations.

Marloth, from long and extensive acquaintance with the Cape flora, has emphasized the view that protection from intense insolation is of great importance in this climate.¹ He points to the large number of leaves which assume the vertical position, and suggests that the screening effect of white hairy coverings in other leaves may be as important as the protection they afford against drying winds.

A large-celled epidermis will act as a screen to an extent which must vary as the height of the cells and perhaps also depend upon the nature of the sap. On the latter point research is required. It is of interest, in this connexion, that solutions of tyrosine have been shown to absorb ultra-violet radiation and so reduce the toxicity of light to bacteria and *Paramoecium*,² and it is not improbable that other substances commonly occurring in plants may be found to have a similar action.

In *Passerina*, the height of the epidermal cells may be important in connexion with the closing of the groove, for the greater the distance between the outer and inner walls the greater is the difference of curvature and the greater therefore will be the effect of the approximation of them. Moreover, the greater the height of the cells, the greater the leverage at the disposal of the cohesion tension drawing the lateral walls together.

Other Anatomical Features.

Raised Stomata. The stomata, as already described, are raised into

¹ K. Marloth: Die Schutzmittel der Pflanzen gegen übermässige Insolation. Ber. d. D. Bot. Ges., xxvii, 1909, p. 362.

² F. J. Harris and H. S. Hoyt in Univ. of California Publ. in Path., ii, 1910, pp. 245-50; see Physiol. Abstr., iv, 1919, p. 203. Entry 1822 also i. p. 686. Entry 2751.

the groove among the hairs (Fig. 3). The effect is to bring the pore slightly nearer the mouth of the groove and to provide a short tube within. In neither case can the effect on diffusion be important. When the leaf-groove is narrowed, however, the inner epidermis is thrown into folds, and I incline to the view that the slightly raised position of the stomata prevents mechanical closure of the pores. It may also secure greater freedom of opening, as compared with stomata in which the guard cells have to push directly against adjacent cells supported by the rest of the epidermis, as well as reducing to a minimum any mutual interference of adjacent stomata.

Fibres. The fact that in *P. filiformis* the fibres interrupt the palisade layer outside the principal bundles has already been mentioned. The fibres extend farther, however, spreading laterally between the epidermis and

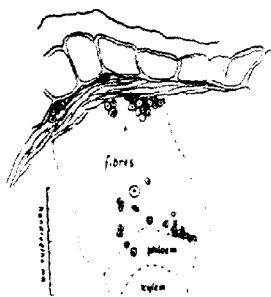


FIG. 6. Section through median bundle of leaf of *P. filiformis*, showing lateral wandering of fibres hypodermally. The fibres drawn near the phloem were the only ones that stained yellow with chlor-zinc-iodine.



FIG. 7. Fibres of ordinary form from minor vein of young leaf of *P. filiformis*.

palisade tissue, where they are seen in transverse sections cut in various directions (Fig. 6). This feature is more strongly marked towards the tip, less strongly towards the base of the leaf.

The small size of the leaf and very small diameter of the fibres (5-10 μ) make it difficult to follow the wanderings of the fibres in sections. Boiling for a few minutes in Schulze's macerating solution makes it possible, however, to remove the epidermis from both sides, and preliminary observations have been made in this way.

In young leaves, associated with the minor veins, fibres of the usual type can be seen, slender and tapering to a point at both ends (Fig. 7). Near the tip in young leaves and extending towards the base in older leaves is found a sub-epidermal sheath of wandering fibres quite different in character. Their tips are often slightly swollen and club-shaped. Their growth is clearly apical. Evidence of similar growth is found deeper in

596 *Thoday.*—*Behaviour during Drought of the Leaves of two*
the leaf associated with the bundles, as well as hypodermally between the
bundles.

Figs. 8 and 9 illustrate these points. In Fig. 8 the irregular course

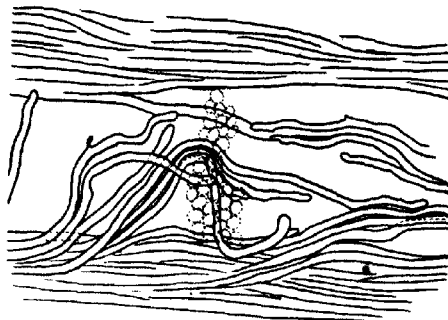


FIG. 8. Hypodermal wandering fibres between two veins. Underlying palisade cells dotted.

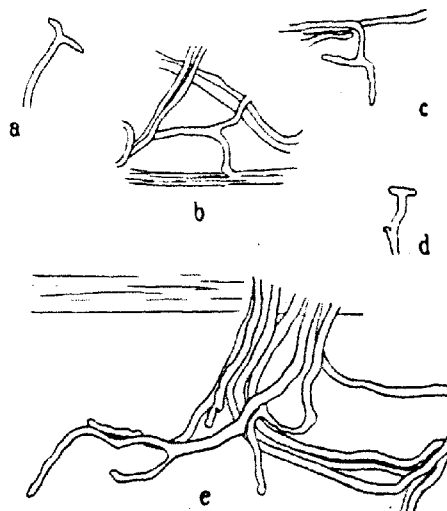


FIG. 9. a-e. Branching hypodermal fibres.

of the hypodermal fibres is shown, between the bundles from which they
are wandering in different directions. Part of the palisade tissue is shown
underlying these fibres.

It is probable that the fibres sometimes branch. Incipient branching
is easy to observe (Fig. 9). Clear evidence of both arms continuing

development is more difficult to obtain as the fibres soon become closely interwoven. The clearest case found is shown in Fig. 9, b.

In *P. cf. falcifolia* fibres are not found except in association with the vascular bundles. Nevertheless, at the free endings of the finer veins, fibres are found with club-shaped tips, suggesting that they have spread by similar hyphal growth from the larger veins (Fig. 10).

The origin of these peculiar hyphae has not yet been observed, and their development requires further study. It seems not unlikely that hyphal growth begins at the leaf apex and that reinforcement of the strands of fibres in both species, as well as the formation of the hypodermal sheath in *P. filiformis*, is brought about in this way. All that can be definitely stated at present is that the hypodermal sheath is formed at a very early stage near the apex of the leaf.

Most of the fibres have cellulose walls stained strongly purple with chlor-zinc-iodine. Relatively few are stained yellow, and these occur scattered near the phloem (Fig. 6). Even these give no appreciable coloration with phloroglucin and hydrochloric acid, and only a very faint yellow with aniline sulphate. They are therefore only slightly lignified.

It might be expected that the hypodermal fibrous sheath of *P. filiformis* would affect the closing mechanism, but I have not been able to obtain any evidence of this from a comparison with *P. cf. falcifolia*. Shoots of the latter collected in spring transpire at first more rapidly than those of *P. filiformis*, but this is probably due principally to the leaves spreading more widely, exposing the grooves.

The data already given, showing the close correlation between closure and water content in shoots collected without discriminating between the two species, indicate that the water content at which closure was complete was the same in both. The significance of the fibrous sheath is a problem, however, on which further evidence is required.

Juvenile Leaves.

Seedlings and young plants have been found belonging to *P. filiformis*, the adult leaves having the palisade layer interrupted and the hypodermal sheath of wandering fibres. Immediately above the cotyledons for a variable distance the leaves are quite different in character (Figs. 11 and 12), being only slightly concave, glabrous, and more or less glaucous. The lower ones

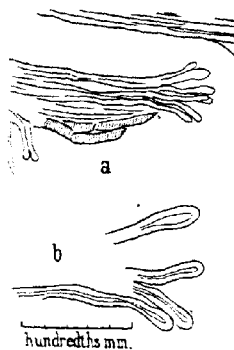


FIG. 10. a. Ultimate vein ending freely, from shade leaf of *P. cf. falcifolia*. The strand of fibres projects like a brush beyond the vascular tissue. The club-shaped tips are shown on a larger scale in b. In a (left) two fibres show abortive wandering.

may show interruption of the palisade layer by fibres, often only in the lateral veins, and extending for a variable distance from the tip. The fibres appear to be, at least the majority of them, of the ordinary tapering form. In leaves higher up, but still juvenile, lateral wandering of fibres from the strands associated with the vascular bundles is already found.

Stomata occur only on the concave upper side. They are not raised, but, on the contrary, are sunk below the surface of the epidermis, and flush with the inner walls (Fig. 12).

The majority of the epidermal cells have thick mucilaginous inner walls (Fig. 11) which normally occupy a volume about equal to that of the

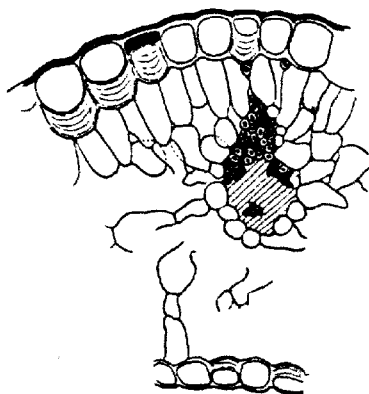


FIG. 11. Middle part of section of juvenile leaf of *P. filiformis*. Epidermis with mucilaginous inner walls; a few fibres wandering longitudinally from the bundle.

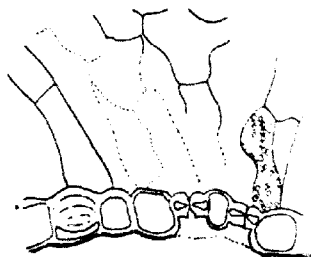


FIG. 12. Portion of section of juvenile leaf of *P. filiformis*, showing sunk stomata in upper concave epidermis.

lumen. These cells are very similar in appearance to the epidermal cells of *Erica* spp. According to Supprian,¹ mucilage is found in the epidermis of *Passerina cricoides*, Linn., but not in other species of the genus. It is found, however, in some species of most of the genera of Thymelaeaceae, including *Anthrosolen* and *Chymococca*, both closely allied to *Passerina*. Preliminary observations of my own point to a wider distribution of this character in *Passerina* than Supprian recorded, but definite statements are necessarily deferred until the species are satisfactorily identified.

In leaf form, absence of groove and woolly hairs, sunk stomata, and mucilaginous inner walls of the epidermal cells the juvenile leaves of *P. filiformis* approximate to those of many other members of the Thyme-

¹ Beiträge zur Kenntnis der Thymelaeaceae. Engler's Bot. Jahrb., xviii, 1894, p. 310.

laeaceae. They represent, therefore, in all probability, a more generalized ancestral type, the ericoid adult leaf being highly specialized.

The transition from juvenile to adult leaves is often remarkably sharp, at one node typical juvenile leaves being borne, at the next typical adult leaves. Occasionally, however, transitional forms are found, with a few woolly hairs towards the tip, which is more involute than in the normal juvenile leaf. But in such cases the stomata, so far as I have observed, are still of the sunk type. The transition is, on the other hand, more often gradual in respect of mucilage. The first adult leaves have a number of epidermal cells on the outer flanks with thick mucilaginous inner walls, and cells may be found here and there in leaves much higher on the young plant which still show the same feature. The transition is also gradual in the degree of development of fibres. The upper juvenile leaves show the characteristic hypodermal sheath of fibres spreading laterally from the veins, and the palisade layer is interrupted at the midrib as well as at the two chief lateral veins.

In reference to the gelatinous contents of the epidermal cells of the

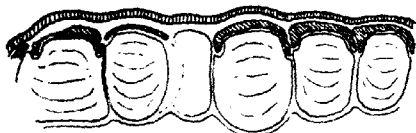


FIG. 13. Dead cells from epidermis of juvenile leaf, showing swelling of mucilage and collapse of lumen. Cuticle and brown contents shaded.

adult leaf, it has been suggested that one advantage lies in the hindrance offered to complete collapse of the cells, if, as sometimes happens in the older leaves, they die prematurely. The mucilage in the epidermis of older juvenile leaves has still more conspicuously a similar action. In dead cells the mucilage swells till it occupies nearly the whole volume of the cell and compresses the lumen with its brown contents into a very small space at the top of the cell (Fig. 13).

Juvenile leaves in P. cf. falcifolia. Seedlings of this form I have seldom found. The lower juvenile leaves are very similar to those of *P. filiformis*, but agree with the adult leaves of the species in having a continuous palisade tissue. Fibres are in fact feebly developed. The number of examples seen is too few for safe generalization, but the mesophyll suggests a shade leaf, with the palisade tissue less strongly developed and the spongy tissue more lacunar than in *P. filiformis*. Other observations of a preliminary nature on the distribution of the two species also point to *P. cf. falcifolia* being a species relatively tolerant of shade, whereas *P. filiformis* grows best in the open, fully exposed to the sun.

On a recently burned slope I have found juvenile shoots of *P. cf. falcifolia* growing from the bases of old charred stems. These, while similar in structure to the juvenile leaves of the seedling, often reached a much larger size, sometimes 2 mm. or more in width, whereas the seedling leaves do not exceed 1 mm.

As in *P. filiformis*, the transition to the adult form is abrupt in respect of grooves, hairs, and stomatal structure.

Cotyledons. The expanded cotyledons of *P. filiformis* have yet another type of structure. Unlike the juvenile and adult leaves, they are convex above, and the palisade tissue, which is feebly developed, is on the upper, not the under side. The stomata, on the other hand, as in the other types, are confined to the upper side of the cotyledons; but they are neither raised nor sunk. Cells with mucilaginous inner walls are found in both the upper and the lower epidermis.

SUMMARY.

The structure and behaviour in drought of the ericoid leaves of two Cape species of *Passerina*, *P. filiformis*, Linn., and *P. cf. falcifolia*, C. H. Wright, are described.

1. The woolly grooves are on the upper side of the leaves. As the water content falls in the dry summer season the edges of the leaf become more and more approximated until in dry situations the groove is completely, even tightly, closed.

2. The water content of shoots with leaf-grooves closed ranged, early in March, 1920, i.e. in the latter part of a very dry summer, from 34 per cent. down to as low as 25.7 per cent. The water content of fully turgid shoots at this time was only 47 per cent. At the end of the winter rainy season (October) the water content of shoots with new growth was about 60 per cent.

3. Evidence is adduced for attributing closure to cohesion forces developed in the outer epidermis, the cells of which are large and deep, and are provided with a very thick cuticle. As they contract with loss of water their lateral walls are thrown into bellows-like folds.

4. The mesophyll, however, contracts in volume during closure at least as much as the epidermis, and the palisade tissue may show fine bellows-like foldings of the walls. Reasons are given for regarding water storage as at most a subsidiary function, of little importance in prolonged drought, and other advantages of a deep-celled epidermis are considered, especially in relation to the closing mechanism and screening from intense insolation.

5. Anatomically, the leaves of the two species agree in most points, including stomata raised in the groove. They differ in the degree of development of the fibres, which in *P. filiformis* interrupt the palisade tissue

outside the chief bundles and spread laterally under the outer epidermis. In surface view, in macerated preparations, the hypodermal fibres are seen wandering like hyphae between the bundles, taking an irregular course, growing apically and occasionally branching. Indications of similar apical growth were found also in the other species, where there is, however, no hypodermal sheath of wandering fibres and the palisade is continuous.

6. The seedlings of both species have juvenile leaves which are slightly concave but not grooved, and are glabrous, with a waxy bloom. The stomata are not raised but sunk, and most of the epidermal cells have thick mucilaginous inner walls, as in adult leaves of species of most genera of the Thymelaeaceae.

7. The cotyledons of *P. filiformis* are convex above, have palisade tissue on the upper side, not as in the juvenile and adult leaves on the under side, and the stomata, though confined to the upper side, are neither raised nor sunk.

The Gametophytes and Fertilization in Laminaria and Chorda. (Preliminary Account.)

BY

J. LLOYD WILLIAMS.

IN the study of artificial cultures of germinating spores of members of this group of Algae, one always observed the presence of a much smaller brown alga along with the undoubted cells or cell-chains that gave rise to the laminarian plants. As the terminal cells of the branches of the smaller alga were frequently found to be empty, the idea suggested itself that they were the antheridia of the male gametophyte.

Until recently all attempts at verifying this conjecture by observing the liberation of the contents and the process of fertilization failed. To Sauvageau belongs the credit of finding the first piece of evidence in favour of the correctness of the above suggestion. Although he, also, failed to find actual liberation of male gametes and fertilization, he was lucky enough to find in *Saccorhiza* abnormal cases of germination of zoospores within liberated but unripe sporangia. In these cases the resulting growths were of two kinds, and similar in every respect to the two always found in normal cultures. This made it almost certain that the smaller alga, which had appeared so puzzling, was also derived from zoospores of *Saccorhiza*, and consequently must be the male gametophyte. The writer of this article did not feel himself justified in publishing his own detailed study of the group until he had seen and studied the actual liberation of antherozoids and the process of fertilization. That this is a very difficult task will be evident when we consider the microscopically small size of the gametophytes, the fact that all the stages must be followed in artificial cultures, and that, in consequence of the time taken in maturing (varying from a few weeks in *Laminaria* to many months in *Chorda*), the sexual cells are liberated, one or a few at a time, with long intervals between. It will be evident that the chances are very much against one being fortunate enough to see these stages at the critical time. Even continuous observation would not suffice to guarantee success, for fertilization may be taking place on a slide while it is being examined under the microscope, yet owing to the smallness of the objects the observer may miss it completely. The

cultures, especially if they are old, often have numbers of monads similar in shape and size, and even in the lateral attachment of the cilia, to phaeophycan zoogametes; in some cases ectocarpoid reproductive bodies occur; this makes it very difficult to be certain of the identity of laminarian antherozoids without having witnessed their liberation.

Over a twelvemonth ago the writer was fortunate enough to be able to fill the gaps in the chain of proofs of the correctness of the theory explained above by observing the liberation of antherozoids, by securing fixed and stained preparations showing the fusion of the sexual nuclei of the fertilization, and, a little later, by actually witnessing the process of fertilization. In response to the urgent requests of botanical friends, a preliminary account of the phenomena is now published, leaving detailed descriptions and drawings to a further series of papers.

The earlier stages in the development of the sexual generations are now sufficiently well known to make it needless to do more than give an outline of the course of events. Although the other British Laminariaceae have been studied, this account will deal only with *Laminaria* and *Chorda*.

The pear-shaped zoospore, with its curved chromoplast and its pair of lateral cilia, after a short period of activity comes to rest, becomes spherical, and invests itself with a wall. A tube then grows out from one side of the spore, the chromoplast divides in two, and they, together with the greater portion of the cytoplasm, travel into the germination tube; the nucleus, however, remains behind in the spore-case. The distal end of the tube swells until it becomes much bigger than the original spore; at the same time the nucleus divides, and one of the two daughter nuclei migrates into the enlargement. A wall separates it and most of the original spore constituents from the basal bulb and germination tube, which are now empty save for the second nucleus, now rapidly degenerating, together with occasional traces of cytoplasm. In *Laminaria* the first division of the nucleus is accomplished as a rule in the spore-case, rarely in the tube. In *Chorda* the reverse is the rule. In *Chorda* also the tube is generally much longer than in *Laminaria*. In these two plants there is no perceptible difference between the early stages of the two kinds of gametophytes.

In *Laminaria* vigorous cultures show differentiation of gametophytes in a week or less, and new sporophytes may appear in a fortnight. In slow cultures the time taken by the process may be very much longer. In *Chorda* no fertilization has yet been observed under three months, and very often the time occupied is six months or more.

The young female gametophyte of *Laminaria* sometimes produces a new sporophyte without further division; in other cases a cell-chain is produced which may even show some amount of branching. These

growths are very irregular in form and always creep along the bottom. The male gametophyte behaves very differently. It is much smaller than the female: it divides up into much smaller cells, which are more numerous than in the female gametophyte. Sometimes all the cells are short, and the plant is compact; in others the branches are thin and slightly elongated. Any cell can function as an antheridium. The process commences in the end cells, which become much paler; the wall at the apex of the cell swells greatly and forms a cap or beak; this eventually bursts, and the single antherozoid swims away; ultimately all the cells of the gametophyte become emptied of their contents. One of the first signs of the approaching maturity of a culture is the appearance in it of a few empty terminal cells with openings at their distal ends. Soon after this, the oogonia begin to be differentiated. The one-celled gametophytes, or one or more cells in a multicellular gametophyte, elongate slightly, and become pear-shaped. The wall covering the narrow end becomes very greatly thickened and differentiated into three layers, the middle one showing a laminated structure. The chromoplasts in the narrow end are crowded together, with their long axes parallel to that of the oogonium, and there are evidences of strong internal pressure. A narrow fissure appears in the innermost layer at the apex of the oogonium; this extends outward, but very often before its completion the outer layer bursts and gapes open; the contents are then pressed out through the opening, now much widened. When the whole protoplast has emerged, the thick walls close elastically, and the only evidences of the aperture are a very faint median line and a rim or collar embracing the base of the protoplast, which thus appears seated in a very shallow cup.

One remained long without any clear evidence as to the particular stage at which fertilization was effected: though not probable, it was possible that the antherozoid effected an entrance into the oogonium through the swollen, mucilaginous apex, and that this may have formed the impulse bringing about the liberation of the egg. Now, however, one is able to decide the question, for the eggs have been seen emerging, and, in the cases where antheridia matured at the same time, they have been seen surrounded by active antherozoids. Thus, there can now be no doubt that there is here a differentiated oogonium, and that the single egg produced by it is fertilized after emergence. Fixed and stained preparations often show the gametic nuclei in various stages of fusion. These nuclei cannot be the result of the first division of the oospore nucleus, for, as will be explained later, the appearances presented at that stage are totally different. In one instance the egg had not completely emerged; and its nucleus, elongated and somewhat deformed by pressure, was actually in the narrow neck of the oogonium while the male nucleus had already entered the free apex of the egg.

In many cases the eggs are observed to have partly disintegrated after emergence. It is not known at present whether this is due to bursting, or to absence of fertilization. While most of the new plants remain attached to the empty oogonium, many become detached and float away. Under natural conditions this probably occurs far more frequently.

The new sporophyte grows rapidly; it is positively heliotropic, so that its direction of growth is quite different from that of the gametophyte, and it is far more regular in its sequence of divisions and in its resulting form. If a preparation be stained with a very dilute solution of polychrome methylene-blue the gametophytes are left uncoloured, but the young sporophytes become at once very prominent owing to the deep pink or purple colour of their cell-walls. The deeper colour occurs in the younger parts; here the minute structure of the wall is very interesting. Further description must be deferred to a future paper.

The first rhizoid makes its appearance early in the form of a rapidly growing, very long protrusion from the basal cell of the sporophyte. This often applies itself in its early stage to the outer surface of the empty oogonium, but, so far, the writer has never observed a case where it entered the oogonium.

The corresponding stages in *Chorda*, through agreeing with the above in their main features, present interesting differences of detail. The gametophytes are many times bigger than those of *Laminaria*. They branch again and again in a very irregular manner until they appear like so many miniature bushes. While there is a distinct difference of size between the two kinds of gametophyte, it is never so extreme as in *Laminaria*. Owing to the long time taken to mature, there is a greater uncertainty and consequent difficulty in securing the critical stages than in the former, and, as it is impossible to obtain pure cultures, the long delay enables other organisms to grow and multiply. A still greater difference is seen in the mode of emergence of the egg. The oogonial wall swells but a very little; the outer layer bursts, and the contents, instead of completely emerging, grow out still enclosed in the extensible inner wall of the oogonium. The emergent part is thus still in communication with the oogonial cavity, and, though the bulk of the contents have migrated out of the latter, a few chloroplasts and granules remain in it. Reinke's well-known figure of a *Chorda* germling shows this well, but he failed to observe the torn edges of the aperture, which forms a more or less distinct collar or sheath indicating the position of the former apex of the oogonium. It follows from what has been said above that the anchorage of the new sporophyte to the gametophyte is more secure here than in *Laminaria*. That the enclosing membrane is not very firm is proved by the occurrence of rare cases where the plantlets break off at the collar. For the same

reason the membrane presents no obstacle to the entry of an antherozoid. (A somewhat parallel instance is observable in *Pelvetia*.) The mouth of the oogonium in *Chorda* remains permanently open. The elongation of the young sporophyte is more rapid than in *Laminaria*, and, in consequence of the multiplication of chloroplasts and the development of their pigments not keeping pace with growth, the whole plantlet looks much paler than the gametophyte from which it has arisen.

A study of the cytology of the different phases confirms the conclusions arrived at on morphological grounds, that in the Laminariaceae we have a case of pronounced alternation of generations, with a great reduction in the gametophytes. The cases in *Laminaria* where the gametophyte consists of a single cell separated from the zoospore by a single nuclear division make it easy to adopt the suggestion that the so-called oogonia and antheridia in the Fucaceae are sporangia. The systematic position of the group has to be changed; and we now get rid of the anomaly of regarding the alga which shows the highest advance in histological differentiation as a member of the Phaeozoosporeae having only asexual reproduction. The investigation also furnishes material for further consideration of the much-debated question of the relations of *Chorda* to the other Laminariaceae.

Fossil Coniferous Wood from Kerguelen Island.

BY

W. N. EDWARDS, B.A.

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With Plate XXIII and four Figures in the Text.

THE material described in this paper was mainly collected in 1840 in the vicinity of Christmas Harbour, Kerguelen Island (lat. 49° S.), by Surgeon R. McCormick, who accompanied Sir James Ross on the voyage of the *Erebus* and *Terror* to Antarctic regions, and who bequeathed his geological collections to the British Museum (Natural History) in 1890.

The McCormick collection of Kerguelen woods contains about forty or fifty pieces of various sizes, some of which are obviously very poorly preserved. Fourteen specimens were sectioned by Mr. J. Lomax, and though some were very different in external appearance and mode of preservation, it was rather disappointing to find that they agreed very closely in internal structure, and have all been included in a single species of *Cupressinoxylon*.

There are also a few specimens probably presented by McCormick's colleague, (Sir) J. D. Hooker, some of which were recently described by Prof. A. C. Seward as *Dadoxylon kerguelense* (Seward, 1919, p. 185). The Bryson collection of plant sections in the British Museum, which includes Nicol's specimens and others made many years ago, contains two slides, (51534 and 51748) labelled 'Kerguellans Land', but there is no further information in the register. They are sections of poorly preserved dicotyledonous wood, and seem to have been cut from the same specimen. The Museum collections do not contain, however, any undoubted blocks of fossil dicotyledonous wood from Kerguelen.

Occurrence of the wood. The northern extremity of Kerguelen Island is largely covered with thick beds of basalt. Ross (1847, vol. i, p. 71) states that the basalt 'is upwards of 500 feet thick, and rests upon the older rocks at an elevation of 600 feet above the sea; and it was between these rocks of different ages that the fossil trees were chiefly found, and one exceeding seven feet in circumference was dug out and sent to England'. (This specimen has not been traced.) Ross says further that the wood was found

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'in every stage from that of charcoal lighting and burning freely' when put in the fire, to so high a degree of silicification as to scratch glass. A bed of shale, several feet in thickness, which was found overlying some of the fossil trees, had probably prevented their carbonization when the fluid lava poured over them.' McCormick gives a detailed account (McCormick, 1842, reprinted in Ross, 1847, p. 74) of the occurrence of wood, and states that it was found 'both imbedded in the basalt and in the débris below, or scattered on the surface amongst the fragments of rocks'. He refers to the shale, but says that no remains of leaves could be discovered in it. (His collection contains a few small pieces of sandy shales full of plant impressions too fragmentary for identification, and labelled 'Christmas Harbour'.) Beds of slaty coal were met with at many points under the basalt. Hooker (1847, p. 219) writes: 'Throughout many of the lava-streams are found prostrate trunks of fossil trees of no mean growth, and the incinerated remains of recent ones, so that it seems impossible to reckon the period of time that must have elapsed between the origin, growth, and destruction of the successive forests now buried in one hill.'

The fossil wood was also observed by members of later expeditions to the island, notably the voyages of the *Gazelle* (Studer, 1889, p. 61) and the *Challenger* (Murray, 1885, p. 349), and the German South Polar Expedition of 1901-3 (Philippi, 1908, p. 197). The wood brought back by the *Gazelle* from Christmas Harbour was examined microscopically by Goeppert (1881, p. 28) and by Beust (1884, p. 10). The former signalized the presence of araucarian wood, but without description or illustration, and, as Gothan (1908, p. 13) says, it is doubtful whether Goeppert's *Araucarites Schleinitzii* et *Hookeri* refers to one species or to two, and both are really *nomina nuda*. However, Seward (1914, p. 11) stated in 1914 that 'the examination of sections cut from a piece of petrified wood in the British Museum, obtained by Sir J. D. Hooker, enables me to confirm Goeppert's statement as to the occurrence of araucarian wood', and later these sections were named *Dadoxylon kerguelense* (Seward, 1919, p. 185). Beust in 1884 named some of the Christmas Harbour wood *Cupressinoxylon antarcticum* (Beust, 1884, p. 12), but he was only able to examine a few small fragments. He described it as having separate bordered pits in one row, abundant resin parenchyma, and uniseriate rays 1-8 cells high. The specimens here described from the same locality apparently belong to the same species.

The only other paper on the internal structure of Kerguelen wood is by Crié (1889, p. 8), who described as *Cupressinoxylon kerguelense* some well-preserved pieces of silicified wood which had been sent him from London by Gardiner and Etheridge. His description is, however, wholly inadequate, and his illustrations are poor. He states that *C. kerguelense* appears to differ from all hitherto described species, and then gives a reference to Beust's paper, but does not say wherein the differences lie. According to Crié, the

secondary wood contained here and there single scattered resin cells (as seen in transverse section; his figures of longitudinal sections show no parenchyma), and the tangential walls of the tracheides are pitted, whereas in the McCormick specimens there is abundant resin parenchyma and no tangential pitting. Moreover, Crié shows the tracheide pits in contact in a single row, and it is even possible, as Kräusel (1919, p. 208) suggests, that the wood was really araucarian.

Age of the specimens. The Tertiary date of the basalts has generally been assumed, partly on the evidence of the fossil woods (Philippi, 1908, p. 197). Though these may well be of Tertiary age, however, they cannot be considered as absolutely definitive, nor is it possible to fix the horizon any more exactly on geological grounds. Seward (1914, p. 208) makes a comparison with the plants preserved in the Tertiary basalts of north-west Europe, and, referring to the resemblance between the basaltic hills of Kerguelen and of South Victoria Land, suggests an extensive basaltic outpouring in Tertiary times. Philippi further remarks that the weathering and changes that have taken place in the basalt point to the outflow being of earlier rather than later Tertiary date.

Kräusel's reference (1919, p. 208) to the age of *C. kerguelense*, Crié, as 'Trias ? (Tertiar ?)' is doubtless an error, for Crié considered the wood to be Tertiary.

Cupressinoxylon antarcticum, Beust.

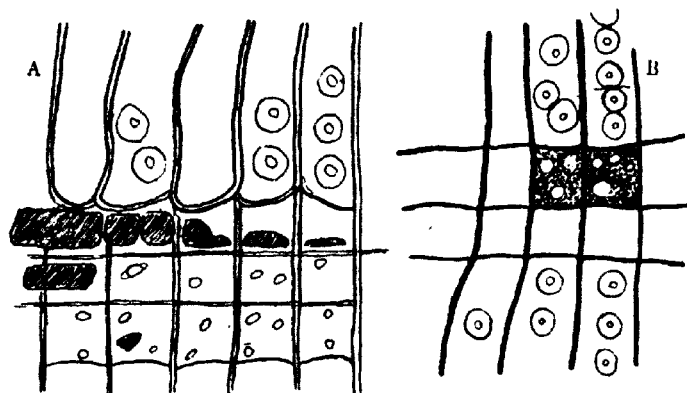
Description: Annual rings well marked; resin canals entirely absent; resin parenchyma abundant; bordered pits usually in one row and usually separate and scattered, rarely in two rows, opposite and separate or adjacent, occasionally in contiguous pairs and slightly flattened, the pairs being vertically or obliquely placed; rims of Sanio present, frequently attached to the margin of the pit; tangential walls of tracheides unpitted; medullary rays uniseriate, very rarely partly biseriate. 1-15 cells in height (commonly 1-6), frequently containing resin; abietinean pitting absent; field pitting not usually preserved, but occasionally 1-4 rather small and apparently simple pits present.

Locality: Christmas Harbour, Kerguelen Island, lat. 49° S.

Age: ? Tertiary.

General remarks: All the specimens examined are of mature wood. Some of the stems being of considerable girth, and no young twigs have been seen. The annual rings vary from 10 to 40 cells in width in different specimens, and the spring wood is usually distorted and crushed. The preservation is frequently very poor, and the tracheides often show the spiral markings which are due to decay. The occasional occurrence of contiguous and partly alternate pits is not uncommon in both living and fossil non-araucarian Conifers.

The field pitting (see Text-fig. 1) is rarely distinguishable, and when preserved is sometimes similar to that figured by Stopes in *C. lucombense* (Stopes, 1915, p. 183, Fig. 52) and by Seward in *C. orientale* (1912, Pl. V, Figs. 73, 75). In all three cases the irregular appearance of the field pitting is probably due to partial decay and enlargement of the original pits. In Slide V. 13615 *d* (see Text-fig. 1, B) from one to three small roundish or oval and apparently simple pits can be seen at one point. Some of these pits are obliquely placed and narrowly elliptical, but it is difficult to distinguish a border, perhaps owing to decay before preservation. This pitting is somewhat like the 'podocarpoid' type (Gothan, 1905, p. 48) and perhaps the Kerguelen species may belong to *Podocarpoxylon*. It is, however, impossible to make exact comparisons with living species, or to define the



TEXT-FIG. 1. *Cupressinoxylon antarcticum*. Radial longitudinal sections, showing field pitting of the medullary rays. A, Slide V. 10291. B, Slide V. 13615 *d*.

precise position of this fossil, which is best included in the genus *Cupressinoxylon*, using the term in a wide sense.

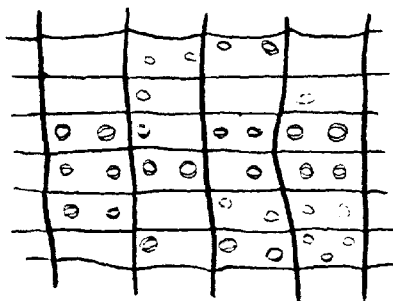
The field pitting in Beust's wood was apparently fairly well preserved, and in some of his figures (Beust, 1884, Pl. IV, Fig. 7) the pore seems to be obliquely placed, thus showing an approach to the podocarpoid type. Kräusel (1919, p. 206) thinks that it may belong to the *Juniperus* group (*Fitzroya*?), but Beust's figures scarcely warrant this conclusion. I have seen no trace of *Juniperus* pitting in the present material, though the state of the wood precludes one from being absolutely certain of its absence.

Among the few known examples of *Cupressinoxylon* from the southern hemisphere, reference must be made to *C. Hookeri*, Arber (1904). On the ground of Arber's description of 'a small simple pit on the radial walls' this species was referred by Gothan (1908, p. 7) to *Podocarpoxylon*, and Seward (1919, p. 211), stating that 'in some places a single fairly large

simple pit occurs in the field', includes the species doubtfully in the comprehensive genus *Mesembrioxylon*. As far as my observations go, wherever the field pitting is preserved there are almost invariably (at any rate in the spring wood) two small pits with a broadly elliptical horizontal pore (Text-fig. 3). The type sections are rather thick in places, and bordered pits on the tracheides overlying the rays might appear to be single pits in the field, but, since the wood is very transparent, the pair of smaller pits as just described may frequently be seen by focusing up and down. The Museum collection contains many fragments from the large specimen (Slide V. 332), and, as they split up very easily, it is possible to separate out the individual medullary rays. Such rays usually show faint vertical lines marking the



TEXT-FIG. 2. *Cupressinoxylon antarcticum*. Tangential longitudinal section showing a partly bisinate ray with adjoining parenchyma. Slide V. 13616c.



TEXT-FIG. 3. *Cupressinoxylon Hookeri*, from Tasmania. Radial longitudinal section to show medullary ray pitting. Slide V. 332d.

lines of contact of the tracheides, and the field thus marked off usually contains two small pits in the early wood, or a single one in the late wood; the pore is more or less circular, but never vertically elongated. It therefore seems best to retain this species in the genus *Cupressinoxylon*. It differs in several respects from the Kerguelen species, notably in the height of the rays and the tangential pitting of the tracheide walls as well as in the field pitting.

C. Hookeri appears to resemble fairly closely *C. Patagonicum*, Conwentz (1884, p. 441), which is described as having one or two round or elliptical pits in the field. There are no other well-preserved examples of *Cupressinoxylon* from the Southern Hemisphere, for *C. latiporesum*, Conwentz, is included by Gothan in the genus *Phyllocladoxylon*.

Dadoxylon kerguelense, Seward.

Seward's description (1919, p. 185) is as follows: 'Annual rings narrow, often 15-20 tracheides broad, the summer wood being frequently represented by only two rows of elements. There are one or two rows of bordered pits on the radial walls of the tracheides, contiguous, alternate, and often slightly flattened.' This description is based on three old and very thick slides (V. 8388-V. 8390) which have 'Kerguellan, Ross' scratched on the glass, and the figures were made from the radial section (V. 8389). These sections were cut from a block of compact, black, silicified wood (V. 5867) bearing an old label 'Kerguellan, Sir J. Ross'. Three more sections (V. 5867 *a*, *b*, and *c*) have recently been cut from this piece which show the structural details much more clearly.

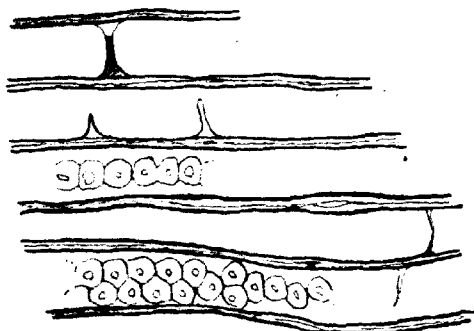
* The growth rings are very distinct to the naked eye in transverse section, and are much closer together than in the *Cupressinoxylon* above described. There are about 18 rings per centimetre, and their curvature indicates a trunk of considerable girth. The diameter of the block in a radial direction is about 10 cm., so there are about 180 rings in this fragment alone, which consists entirely of secondary wood. The rings have a somewhat root-like character.

The preservation is good, and there has been no crushing of the tissues. The tangential walls of the tracheides are unpitted. The medullary rays are uniseriate, 1-11 cells high, and there may be as many as nine oblique pits in the field. The most striking feature, however, is the distribution of the resin in the tracheides, which closely resembles that described by Dr. Stopes (1914) in *D. novae-Zelandiae*, and by R. B. Thomson (1914) in various recent and fossil araucarians. Plates or spools of resin may be seen in both radial and tangential sections in the tracheides adjacent to the rays, and in some cases these tracheides appear to have thickened walls. The transverse section has a striking appearance: in parts of the wood the resin seems to be absent, and while in some patches the resin is confined to the tracheides next to the rays, in other regions practically every tracheide is filled with a dark brown, presumably resinous substance (see Pl. XXIII, Figs. 4 and 5). In the longitudinal sections isolated tracheides are to be seen entirely filled with resin, which is found also in the medullary ray cells.

The spools in the tracheides are sometimes so reduced as to give the appearance of septa (Text-fig. 4), and in fact it is very difficult to say whether or not actual septa or trabeculae were present. In one or two cases these apparent septa are arranged to some extent in radial rows, and it seems possible that the septa described by Gothan (1908, p. 10) in *Dadoxylon pseudoparenchymatosum* from Seymour Island (lat. 64° S.) were reduced resin plates, for they occur in the same position, in connexion with

the rays. Gothan discusses in detail the significance of the supposed septate tracheides, though without reaching any definite conclusion. He does not consider that they are resin parenchyma because of the absence of any resin content, though resin is often present as a dark mass in the ray cells. The appearance of the transverse section, however, as described by Gothan ('eine Anzahl von Zellen, die scheinbar mit dunklerem, bräunlichem Inhalt erfüllt scheinen'), supports the idea that the septa are really resin plates. The fact that the resin was so reduced in the tracheides and not in the ray cells is not surprising when one considers the variation in the amount and distribution of the resin in *D. kerguelense* and other araucarians.

Though the non-committal name *Dadoxylon* is used for the Kerguelen species, it agrees closely in structure with living araucarians, and among



TEXT-FIG. 4. *Dadoxylon kerguelense*. Radial longitudinal section, showing thin plates and projections of resin. Slide V, 6867/6.

fossil species from the Southern Hemisphere it is very near to those already mentioned from New Zealand and Seymour Island. Gothan indeed calls his wood *D. (Araucaria) pseudoparenchymatosum*, thus indicating its close affinity with living types, though since there seems to be no method of deciding whether any fossil araucarian wood belonged to *Araucaria* or to *Agathis*, it seems better to use the name *Dadoxylon* alone.

Gothan's species differs from *D. kerguelense* chiefly in the point of resin distribution. It is of Tertiary or perhaps Upper Cretaceous age, while *D. novae-Zelandiae* is supposed to be mid-Cretaceous. The latter and *D. kerguelense* agree in most characters, including the presence of resin spools, but in *D. novae-Zelandiae* the growth rings are better marked, and the late wood is several cells broad; the medullary rays are also only 1-7 cells high instead of 1-11, and the field pits, according to Dr. Stopes, are 5 or 6 in number, while there are frequently more in *D. kerguelense*.

These, however, are probably not constant specific characters. Another Tertiary species, *Dadoxylon Doeringii* (Conwentz), from the Oligocene of Patagonia (Conwentz, 1884, p. 448), is not so closely related, for it has much higher and occasionally biseriate rays.

SUMMARY.

An examination of fossil wood from Kerguelen Island in the British Museum (Natural History) has revealed the presence of only two species (apart from some rather doubtful dicotyledonous wood), the anatomy of which is described in detail. In the case of *Cupressinoxylon antarcticum*, Beust, the preservation of the minuter features does not permit of an exact comparison with recent genera.

The other species, *Dadoxylon kerguelense*, Seward, closely resembles the wood of living araucarians, and has well-developed resin spools or plates in the tracheides.

These fossils do not give sufficient indication of the precise age of the basalts beneath which they were found, and which are generally regarded as being of Tertiary, and perhaps early Tertiary, date.

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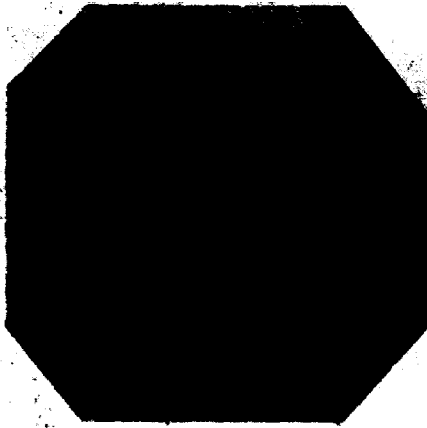
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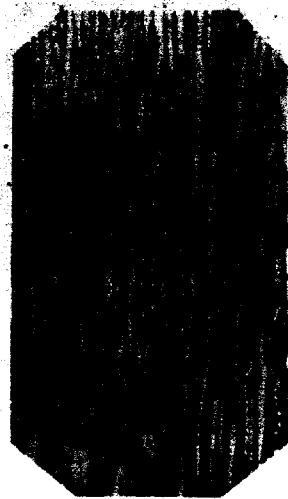
EXPLANATION OF PLATE XXIII.

Illustrating Mr. W. N. Edwards's paper on Fossil Coniferous Wood from Kerguelen Island.

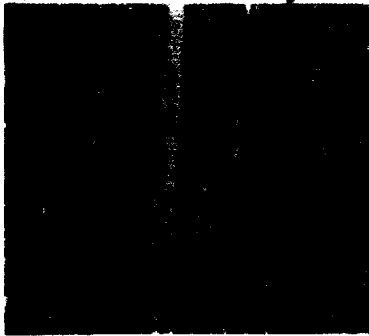
- Fig. 1. *Cupressinoxylon antarcticum*. Transverse section of wood. $\times 50$. Slide V. 13616 a.
- Fig. 2. Same. Radial longitudinal section. $\times 50$. V. 13615 d.
- Fig. 3. Same. Tangential section. $\times 50$. V. 13612 c.
- Fig. 4. *Daloxylon kerguelenic*. Transverse section, showing resin in tracheides adjoining the rays. $\times 30$. V. 5867 a.
- Fig. 5. Same, showing resin-filled area on the right and clear area on the left. $\times 30$. V. 5867 a.
- Fig. 6. Same. Radial longitudinal section, showing bordered pits in one and two rows, and a medullary ray with 3-7 pits in the field. $\times 150$. V. 5867 b.
- Fig. 7. Same. Radial section, showing semicircular blobs of resin in tracheides adjoining the rays. $\times 50$. V. 5867 b.
- Fig. 8. Same. Tangential section, showing resin plates and blobs. $\times 50$. V. 5867 c.
- All the slides are in the Geological Department of the British Museum (Natural History). The photographs were taken by Mr. F. W. Edwards.



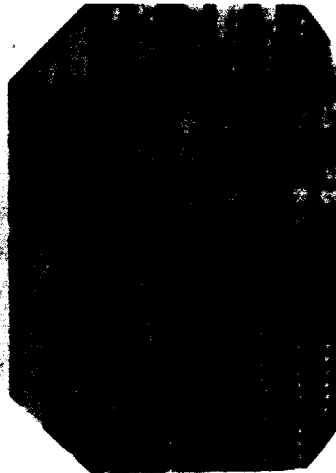
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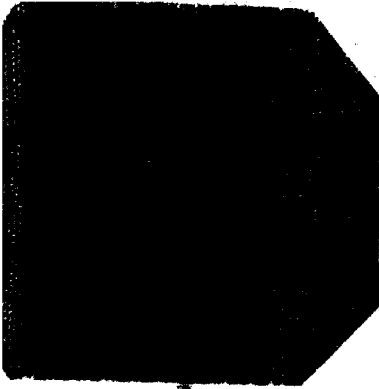


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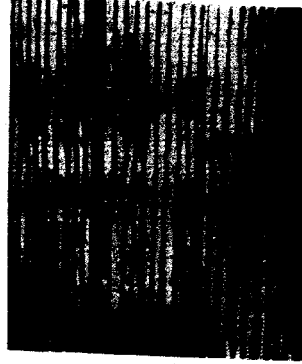


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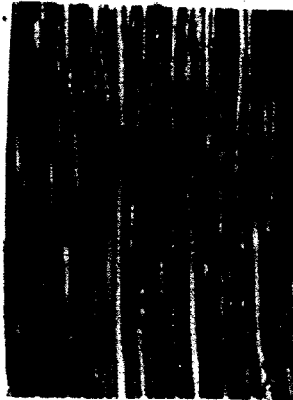
EDWARDS - CUPRESSINOXYLON & DADOXYLON.



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INDEX.

A. ORIGINAL PAPERS AND NOTES.

	PAGE
ARBER, AGNES.—The Leaf Structure of the Iridaceae, considered in Relation to the Phyllode Theory. With sixty-six Figures in the Text	301
BIEWS, J. W.—Some General Principles of Plant Distribution as illustrated by the South African Flora	1
BLACKBURN, KATHLEEN B., and HARRISON, J. W. HESLOP.—The Status of the British Rose Forms as determined by their Cytological Behaviour. With Plates IX and X and five Figures in the Text	159
BOYLE, C.—Studies in the Physiology of Parasitism. VI. Infection by <i>Sclerotinia Libertiana</i> . With Plate XIV	337
BRENCHLEY, WINIFRED E., and JACKSON, VIOLET G.—Root Development in Barley and Wheat under Different Conditions of Growth. With four Figures in the Text	533
BROWNE, ISABEL M. P.—A Fourth Contribution to our Knowledge of the Anatomy of the Cone and Fertile Stem of <i>Equisetum</i> . With Plate XXI and twelve Figures in the Text	427
BUTLER, O.—See SMITH, T. O.	
CAMPBELL, DOUGLAS HOUGHTON.—The Gametophyte and Embryo of <i>Botrychium obliquum</i> , Mühl. With Plate VIII and eleven Figures in the Text	141
CAYLEY, DOROTHY M.—Some Observations on the Life-history of <i>Nectria galligena</i> , Bres. With Plates IV and V	79
CHANDLER, M. E. J.—Note on the Occurrence of <i>Sequoia</i> in the Headon Beds of Hordwell, Hants	457
CUTTING, E. M.—On the Pollination Mechanism of <i>Incarvillea Delavayi</i> , Franch. With three Figures in the Text	63
——— Observations on Variations in the Flowers of <i>Stachys sylvatica</i> , Linn. With five Figures in the Text	409
DANIELS, MARGARET E.—See HOLDEN, H. S.	
DASTUR, JEKANGIR FARDUNJI.—Cytology of <i>Tilletia Triticis</i> , (Bjerk.) Wint. With Plate XX and nine Figures in the Text	399
EDWARDS, W. N.—Fossil Coniferous Wood from Kerguelen Island. With Plate XXIII and four Figures in the Text	609
GATES, R. RUGGLES, and REES, E. M.—A Cytological Study of Pollen Development in <i>Lactuca</i> . With Plates XVI-XIX	365
GREGORY, F. G.—Studies in the Energy Relations of Plants. I. The Increase in Area of Leaves and Leaf Surface of <i>Cucumis sativus</i> . With fifteen Figures in the Text	93
GUPPY, H. B.—The Testimony of the Endemic Species of the Canary Islands in Favour of the Age and Area Theory of Dr. Willis	513
HARRISON, J. W. HESLOP.—See BLACKBURN, KATHLEEN B.	
HATFIELD, E. J.—Anatomy of the Seedling and Young Plant of <i>Macrozamia Fraseri</i> . With Plate XXII and eight Figures in the Text	565
HOLDEN, H. S., and DANIELS, MARGARET E.—Observations on the Anatomy of Teratological Seedlings. IV. Further Studies on the Anatomy of Atypical Seedlings of <i>Impatiens Rhylei</i> , Walp. With ninety-seven Figures in the Text	461
HOLMES, M. G.—A Contribution to the Study of Water-conductivity in Sycamore Wood. With thirteen Figures in the Text	251
HORNE, ARTHUR S.—Phloem Necrosis (Brown Rust Disease) in <i>Hevea brasiliensis</i>	457

	PAGE
JACKSON, VIOLET G.—See BRENCLEY, WINIFRED E.	
JEFFREY, EDWARD CHARLES, and TORREY, RAY ETHAN.—Transitional Herbaceous Dicotyledons. With Plates XI-XIII and five Figures in the Text	227
MOTTIER, DAVID M.—On Certain Plastids, with Special Reference to the Protein Bodies of Zea, Ricinus, and Conopholis. With Plate XV	349
REES, E. M.—See GATES, R. RUGGLES.	
SAHNI, B.—Note on the Presence of a 'Tent-pole' in the Seed of <i>Cephalotaxus pedunculata</i> . With two Figures in the Text	297
SEIFRIZ, WILLIAM.—Observations on some Physical Properties of Protoplasm by Aid of Microdissection. With one Figure in the Text	269
SMITH, T. O., and BUTLER, O.—Relation of Potassium to Growth in Plants	189
STOPES, MARIE CARMICHAEL.—The Missing Link in <i>Osmundites</i> . With Plate II and one Figure in the Text	55
TAYLOR, NORMAN.—Endemism in the Bahama Flora. With one Map in the Text	523
THODAY, D.—On the Behaviour during Drought of Leaves of two Cape Species of <i>Passerina</i> , with some Notes on their Anatomy. With thirteen Figures in the Text	585
THODAY, M. G.—Anatomy of the Ovary and Seed in <i>Gnetum Gneumon</i> , with Notes on <i>Gnetum</i> funiculare. With Plate I and five Figures in the Text	37
TORREY, R. E.—Telephragmoxylon and the Origin of Wood Parenchyma. With Plate III and three Figures in the Text	73
—See JEFFREY, EDWARD CHARLES.	
WALKDEN, H.—The Isolation of the Organism causing Crown Gall on <i>Chrysanthemum frutescens</i> in Britain	137
WATERHOUSE, W. L.—Studies in the Physiology of Parasitism. VII. Infection of <i>Berberis vulgaris</i> by Spores of <i>Puccinia graminis</i> . With nineteen Figures in the Text	557
WELSFORD, E. J.—Division of the Nuclei in <i>Synchytrium endobioticum</i> , Perc. With one Figure in the Text	298
WILLIAMS, J. LLOYD.—The Gametophytes and Fertilization in <i>Laminaria</i> and <i>Corda</i> (Preliminary Account)	603
WILLIAMSON, H. S.—A New Method of preparing Sections of Hard Vegetable Structures	139
WILLIS, J. C.—Endemic Genera of Plants in their Relation to Others. With six Diagrams in the Text	493
WORMALD, H.—On the Occurrence in Britain of the Ascigerous Stage of a 'Brown Rot' Fungus. With Plates VI and VII	125

B. LIST OF ILLUSTRATIONS.

- α. PLATES.
- I. *Gnetum* (M. G. THODAY).
 - II. *Osmundites* (STOPES).
 - III. *Telephragmoxylon* (TORREY).
 - IV, V. *Nectria galligena* (CAYLEY).
 - VI, VII. Brown Rot (WORMALD).
 - VIII. *Botrychium* (CAMPBELL).
 - IX, X. *Rosa* (BLACKBURN and HARRISON).
 - XI-XIII. Herbaceous Dicotyledons (JEFFREY and TORREY).
 - XIV. *Sclerotinia Libertiana* (BOYLE).
 - XV. Plastids (MOTTIER).
 - XVI-XIX. *Lactuca* (GATES and REES).
 - XX. *Tilletia Tritici* (J. F. DASTUR).
 - XXI. *Equisetum* (I. M. P. BROWNE).
 - XXII. *Macrozamia* (HATFIELD).
 - XXIII. Coniferous Wood (EDWARDS).

Index.

vii

6. FIGURES.

FIGURES.	PAGE.
1. Diagrammatic view of section of young ovule of <i>Gnetum Gnetum</i> , 3 mm. long, in which closure of micropyle is just beginning (M. G. THODAY)	38
2. Drawn from mature seed of <i>G. Gnetum</i> , 3 cm. long (M. G. THODAY)	42
3. The apex of another mature seed of <i>G. Gnetum</i> (M. G. THODAY)	43
4. <i>G. funiculare</i> . Cross-section of apex of seed, showing two bands of fibres in outer covering, 4-lobed stony layer of middle covering, and lignified micropylar tube (M. G. THODAY)	47
5. Apex of seed of <i>Bennettites Morierii</i> (from Lignier, 1894, relettered for purposes of comparison) (M. G. THODAY)	50
Diagram of leaf-base of <i>Osmundites Kidstoni</i> , sp. nov., showing the position of the meristele and sclerotic tissues (STOPEs)	57
1. Side view of flower of <i>Incarvillea Delavayi</i> (slightly reduced) (CUTTING)	63
2. A. Anther-lobes of longer stamen. B. Back view of shorter stamen. C. Young stigma. D. Diagrammatic view of flower from above (with a slit in the side). E. Diagrammatic longitudinal section of flower. F. Diagram of anther showing the pad touching the style (CUTTING)	64
3. Pollen-grains of <i>I. Delavayi</i> , A-F, and of <i>I. grandiflora</i> , G-L (CUTTING)	68
1-3. 1. <i>Telephragmoxylon</i> . 2. <i>Brachyoxylon</i> . 3. <i>Picea</i> . Origin of parenchyma (TORREY)	75
1. Method of estimating leaf areas (GREGORY)	93
2, 3. " " " " " "	95
4. Curves of increase in the linear dimensions of a leaf (GREGORY)	101
5. Ditto in area of third leaf, June 1917 (GREGORY)	102
6. Ditto in total leaf surface, March 1917 (GREGORY)	103
7. Ditto, June 1917 (GREGORY)	104
8, 9. Growth in area of cotyledons and leaves under two lamps and one lamp respectively (GREGORY)	108
10. Curve of increase in leaf surface under two lamps and one lamp respectively (GREGORY)	111
11. Equations of curves of closest fit, March, June (GREGORY)	114
12. Showing for each day the actual areas of successive leaves from the cotyledons onwards (GREGORY)	115
13. Curve of increase in total leaf surface, December 1916 (GREGORY)	116
14. Equation of curve of closest fit, December 1916 (GREGORY)	117
15. Equations of curves of closest fit for two lamps and one lamp (GREGORY)	119
1. A. Gametophytes of <i>Botrychium obliquum</i> , Mühl., some with attached sporophytes. B. Young sporophyte with expanded cotyledon, attached to the gametophyte (CAMPBELL)	142
2. A. Median section of an antheridium just before the final division of the spermatocytes. B. Empty antheridium. C. Surface view of antheridium with triangular opercular cell. D. Surface view of antheridium with two opercular cells (CAMPBELL)	143
3. A. Young archegonium. B-D. Older archegonia. E. Mature archegonium, showing apparent ventral canal cell. F. Open, but unfertilized, archegonium (CAMPBELL)	145
4. A. Recently fertilized archegonium containing unicellular embryo. B-D. Older unicellular embryos (CAMPBELL)	146
5. A. Archegonium containing much elongated unicellular embryo. B. First division in the embryo. C. An older stage. D. Two sections of an older embryo (CAMPBELL)	146
6. A-D. Four of a series of sections of a young embryo. E. Three similar sections of an older embryo in which the cotyledon and root are already evident (CAMPBELL)	148
7. A. Two longitudinal sections of an embryo, cut in the plane of the cotyledon. B. Two sections of a similar embryo, cut in a plane at right angles to that of A. C. Somewhat oblique section of an older embryo showing the conspicuous sheath at the base of the cotyledon (CAMPBELL)	150

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Index.

ix

FIGURES.	PAGE
31-35. Leaves of Iris (Sections Xiphium and Juno) (ARBER)	313
36-39. Bifacial leaves of Moreaea (ARBER)	315
40-49. Leaves of Ixioidae and phyllodes of Acacia (ARBER)	318
50-55. Foliated leaves (ARBER)	321
56-66. Leaves of Crocoideae (ARBER)	324
1-9. 1-7. Penetration of the infecting hyphae through the epidermal intercellular spaces. 8. Ditto directly through the lumen of the epidermal cell. 9. Multinucleate mycelium in the tissues of the leaf-sheath (DASTUR)	405
1, 2. Variations in the Flowers of Stachys sylvatica, Linn. (CUTTING)	410
3. " " " " " "	413
4. " " " " " "	415
5. " " " " " "	416
1. Reconstruction of the stele of Cone A of Equisetum sylvaticum (BROWNE)	431
2. Ditto of the stele of Cone B of E. sylvaticum (BROWNE)	431
3. Ditto of the stele of Cone A of E. debile (BROWNE)	433
4. Ditto of immature stele of Cone B of E. debile (BROWNE)	433
5. Ditto of the stele of Cone C of E. debile (BROWNE)	435
6. Ditto of the stele of Cone D of E. debile (BROWNE)	435
7. Ditto of immature stele of Cone E of E. debile (BROWNE)	435
8. Ditto of the stele of Cone A of E. variegatum (BROWNE)	436
9. Ditto of the stele of Cone B of E. variegatum (BROWNE)	436
10. Ditto of the stele of Cone C of E. variegatum (BROWNE)	436
11. Ditto of the stele of Cone D of E. variegatum from below insertion of annulus to a point above level of first whorl of sporangioophores (BROWNE)	446
12. Section of internodal bundle from the axis of the cone of E. limosum, showing continuity of some of the metaxylem with the protoxylem (BROWNE)	447
1-15. 1. 'Seedling A.' A syncytol combining tetrachly at the base of the hypocotyl with extremely intimate syncytoly. 2a, 2b. 'Seedling B.' A syncytol in which the hypocotyl is diarch; 2b shows the venation of the compound lamina. 3, 4. Syncytols showing tetrachly. 5. A syncytol showing a triarch hypocotyl; the tap-root was pentarch. 6a, 6b. Seedling with markedly unequal cotyledons. 7. A syncytol with unequal cotyledons. 8. Large 'Type 2' seedling in which the epicotyl has not developed. 9, 10a, 10b. Salver-shaped amphisyntocytis, 'Seedlings C and D' respectively. 11-14. Goblet-shaped amphisyntocytis. 15. 'Seedling H' (HOLDEN AND DANIELS)	453
16-28. 16-20 'Seedling A', 21-28 'Seedling B'. Both show a fusion of the cotylar midribs, these behaving in transition like a single normal midrib (HOLDEN AND DANIELS)	464
29. Transverse section of stele, showing the origin of a root-pole, <i>de novo</i> (HOLDEN AND DANIELS)	465
30-42. 30-34 show the transition in a 'Type 2' seedling in which tetrachly has resulted from the production of one new pole and the independence of one lateral. 'The new pole does not give rise to a lateral root at the base of the hypocotyl. 35-38 show a 'Type 1' seedling with similar transition features, a, b, c, d poles, represented in the hypocotyl, e pole present from the base of the tap-root onwards. 39-42 show the transition in a 'Type 1' seedling, in which the three hypocotyledonary poles, a, b, c, are reinforced in the tap-root by two additional ones, d, e (HOLDEN AND DANIELS)	468
43-46. A 'Type 1' seedling in which the tap-root has been destroyed (HOLDEN AND DANIELS)	469
47-52. 47-52 show the transition phenomena in a seedling in which the cotyledons are markedly unequal. 53-58 show the transition in a seedling in which cotyledonary inequality and syncytoly are combined (HOLDEN AND DANIELS)	471
59-63. Transition in a 'Type 2' seedling. 60 shows the effect of partial roofing-in of the plumule on the side of the base of the petiole (HOLDEN AND DANIELS)	474
64-66. An amphisyntocytol in which partial roofing-in of the plumule has occurred, and in which the cortex shows splits due to tissue tensions (HOLDEN AND DANIELS)	475

FIGURES.	PAGE
67-74. 67-70 illustrate the transition phenomena in 'Seedling C' and 71-74 in 'Seedling D' (HOLDEN and DANIELS)	476
75-80. A goblet-shaped amphisyecotyl, showing an abnormal type of transition (HOLDEN and DANIELS)	477
81-88. 81-83 illustrate the earlier stages of transition in an amphitrisyncotyl; 84-88, the earlier stages of transition in a tricotyl (HOLDEN and DANIELS)	478
89-97. A 'Type 1' seedling, showing an abnormal type of transition (HOLDEN and DANIELS)	480
1. Distribution in Ceylon of the earlier VR, R, and RR species from Trimen's 'Flora' (WILLIS)	494
2. Diagram showing hypothetical distribution of a wide in New Zealand (WILLIS)	497
3. Map for <i>Ranunculus</i> (WILLIS)	498
4. Number of wides and endemics at each successive zone of one hundred miles: northern invasion (WILLIS)	499
5. Ditto: southern invasion (WILLIS)	500
6. Percentages plotted as curves (WILLIS)	510
Map of the Bahama Islands (TAYLOR)	531
1. <i>Barley</i> , plants grown in pots. Dry weights of shoots, upper curves; dry weights of roots, lower curves (BRENCHLEY and JACKSON)	540
2. <i>Barley</i> , shoot/root ratio (BRENCHLEY and JACKSON)	543
3. <i>Wheat</i> , plants grown in pots. Dry weights of shoots, upper curves; dry weights of roots, lower curves (BRENCHLEY and JACKSON)	550
4. <i>Wheat</i> , shoot/root ratio (BRENCHLEY and JACKSON)	552
1-11. 1. Sporidium germinating on slide and producing a single germ tube with a mucilaginous sheath. 2. Germinating sporidium producing two germ tubes with mucilaginous sheaths. 3. Ditto producing three germ tubes. 4. Ditto producing a vesicle. 5. Ditto producing vesicle with a mucilaginous investment. 6. Sporidium germinating and producing a vesicle which is developing a germ tube above. 7. Ditto germinating on barberry leaf and producing a vesicle. 8. Ditto on barberry leaf, germinating with production of a simple germ tube, the tip of which is adpressed to the surface of the leaf. 9. Ditto attached to leaf and producing a beak-like protuberance at one end. 10. Beak-like projection of sporidium producing an indentation of the cuticle. 11. Outgrowth from sporidium pressing upon the cuticle and forcing up the end of the sporidium (WATERHOUSE)	560
12-19. 12. Sporidium producing a longer beak-like process with its densely protoplasmic tip closely pressed against the cuticle. 13. Ditto producing a style-like infection hypha which has pierced the cuticle. 14. Ditto showing the infection hypha which has penetrated the whole thickness of the epidermal wall. 15. Infection hypha, developed from a germ tube, which has pierced the cuticle. 16. Germination of sporidium has led to the formation of irregular vesicles. An infection style has penetrated the epidermal cell and is beginning to swell at the tip. 17. A later stage of penetration. The vesicle in the epidermal cell has increased in size. 18. Larger vesicle showing three nuclei. 19. Two epidermal cells which have been invaded, showing the branching of the mycelium arising from the infection vesicle. The shrivelled remains of the germinated sporidia are to be seen on the leaf surface (WATERHOUSE)	561
1. Transverse section of protosteles in hypocotyl of young seedling (HATFIELD)	569
2. Seedling of <i>Macrozamia Fraseri</i> (HATFIELD)	569
3. A. Transverse section of seedling above stem apex, showing cotyledon tube and traces, and first three plumular leaves, in two of which leaf-traces are developed. B. Ditto of same seedling at point where fusion of cotyledon tube and of foliage leaves with axis is nearly complete. C. Ditto of same seedling just above the cotyledonary node (HATFIELD)	570
4. Transverse section of cotyledonary node (HATFIELD)	571
5. A. Transverse section of plant just above stem apex, showing bases of five youngest	

Index.

103 RES.

x1
PAGE

- foliage leaves. B. Ditto of same plant taken just *below* stem apex, showing vascular bundles of young foliage leaves in stem (HATFIELD) 573
6. Portion of longitudinal section of stem, just below stem apex, showing the 'growth ring' or procambium, and cells of pith and cortex which have been formed by it (HATFIELD) 574
7. Xylem and cambium of hypocotyl, as seen in transverse section (HATFIELD). 575
8. Young plant cut longitudinally nearly in the median plane, and treated with phloroglucin to bring out xylem (HATFIELD) 579
1. Tracings of adjacent sections from a leaf of *Passerina* cf. *falcifolia* in which the groove was lightly closed; left, in oil, the edges overlapping; right, in water, groove open, tissues expanded (THODAY) 588
2. Diagrams of sections of old healthy leaves of (a) *P. filiformis*, (b) extreme shade plant of *P. cf. falcifolia* (THODAY) 589
3. Part of section of leaf of *P. cf. falcifolia* (THODAY) 589
4. Epidermis of corresponding parts of two adjacent sections: (a) in oil, showing bellows-like foldings; (b) expanded in water (THODAY) 590
5. Outer epidermal and palisade cells from microtome sections of closed leaf, fixed in absolute alcohol and embedded in paraffin. The contents of each epidermal cell (shaded) form a somewhat contracted brown gelatinous mass. Palisade cells with finely-folded walls (THODAY) 592
6. Section through median bundle of leaf of *P. filiformis*, showing lateral wandering of fibres hypodermally. The fibres drawn near the phloem were the only ones that stained yellow with chlor-zinc-iodine (THODAY) 595
7. Fibres of ordinary form from minor veins of young leaf of *P. filiformis* (THODAY) 595
8. Hypodermal wandering fibres between two veins (THODAY) 596
9. a-c. Branching hypodermal fibres (THODAY) 596
10. a. Ultimate vein ending freely, from shade leaf of *P. cf. falcifolia*. The strand of fibres projects like a brush beyond the vascular tissue. The club-shaped tips are shown on a larger scale in b. In a (left) two fibres show abortive wandering (THODAY) 597
11. Middle part of section of juvenile leaf of *P. filiformis*. Epidermis with mucilaginous inner walls; a few fibres wandering longitudinally from the bundle (THODAY) 598
12. Portion of section of juvenile leaf of *P. filiformis*, showing sunk stomata in upper concave epidermis (THODAY) 598
13. Dead cells from epidermis of juvenile leaf, showing swelling of mucilage and collapse of lumen (THODAY) 599
1. *Cupressinoxylon antarcticum*. Radial longitudinal sections, showing field pitting of the medullary rays. A, Slide V. 10291. B, Slide V. 13615 7 (EDWARDS) 612
2. C. *antarcticum*. Tangential longitudinal section, showing a partly biseriate ray with adjoining parenchyma. Slide V. 13616 c (EDWARDS) 613
3. C. *Hookeri*, from Tasmania. Radial longitudinal section to show medullary ray pitting. Slide V. 332 b (EDWARDS) 613
4. *Dadoxylon kerguelense*. Radial longitudinal section, showing thin plates and projections of resin. Slide V. 5867 b (EDWARDS) 615

ERRATA

Page 91, line 37, for Plate V read Plate IV.
" 92, " 1, " Plate VI read Plate V.

